Development of blowflies (Diptera: Calliphoridae) on pig and human cadavers Implications for forensic entomology casework

Whitaker, Amoret

Awarding institution:
King's College London

Development of blowflies (Diptera: Calliphoridae) on pig and human cadavers: Implications for forensic entomology casework

Amoret Philippa Whitaker

School of Biomedical Sciences

Doctor of Philosophy

Abstract

Laboratory studies conducted on developing blowfly larvae (Diptera: Calliphoridae) showed that even small numbers of larvae resulted in an increase in temperature above ambient, and that they were able to retain heat when exposed to a lower ambient temperature. The increase in larval mass temperatures was also demonstrated in decomposition studies carried out on still-born piglets in London, where *Calliphora vicina* was dominant in an urban environment, and *Lucilia sericata* in a rural environment. Oviposition on indoor piglets was not always delayed, but may instead be delayed on outdoor cadavers if the ambient temperature is low.

Decomposition studies were also carried out in Knoxville, Tennessee, on donated human cadavers. A body laid out on the ground attracted communal oviposition and larval mass temperatures up to 45°C. In contrast, a body placed in an unsealed plastic bin resulted in a delay in colonisation of seven days, with Phorids being the first flies to inhabit the cadaver, and blowfly oviposition delayed by ten days.

The dispersal of post-feeding blowfly larvae from human cadavers was observed, and was found to be highly variable in direction, distance and time span. A further study was conducted to determine the length of time that empty pupal cases can remain in a natural woodland environment. The results suggested that depth of puparia and timespan are the important factors, and that after a period of three and a half years, the pupae are disintegrated and more difficult to locate and recover.

Thermal imaging was also demonstrated as a useful tool in locating and measuring increased larval mass temperatures on both pigs and human cadavers. Benefits of using this method are that it is non-invasive and can be used to record and later analyse thermal images.

Decomposition studies were carried out comparing pig and human cadavers. The results showed that the same species of blowfly are attracted to both types of cadaver, and oviposition sites, larval development and decomposition rates are also comparable, supporting the claim for pigs being a good substitute for humans in decomposition studies.

CONTENTS

List of Figures

List of Tables

Acknowledgments

Enormous thanks are due first to my supervisors – without them I would never have got involved in the fascinating field of forensic entomology. Dr Bryan Turner of King's College London agreed to take me on and despite intermittent contact has always been available to offer guidance and advice. Thanks also go to him for funding my trip to Brisbane to the ICE 2004 meeting and for lively discussions at the annual EAFE meetings. Many thanks also go to Dr Barbara Daniel, who very kindly agreed to take me on at a late stage, and has been the key person in getting me over the final hurdles. I would also like to express my gratitude to my external examiners, Dorothy Gennard and Simon Leather, for their very helpful feedback and comments on this thesis.

My main thank you is to Dr Martin Hall, Head of Research of the Department of Entomology, Natural History Museum, London. I approached him with an idea of doing a PhD in Forensic Entomology to which he agreed without hesitation. Being already based at the NHM with a part-time job, the obvious choice was to carry out my PhD from there, so he has been my main supervisor. Martin has also approved funding for my attendance at 20+ conferences during my PhD studies, where I have been able to present my work and make valuable contacts. I also have Martin to thank for trusting and allowing me to take on forensic casework, which means that I already have many cases under my belt and a wealth of experience. I hope to be able to continue to carry out casework under the Natural History Museum consulting group. I should also extend my thanks to John Jackson at the NHM for arranging part-funding of my studies, paying the first five years of my tuition fees. Without this support I would have struggled financially and may well have faltered.

Thanks should also go to those people who have enabled me to carry out my fieldwork. Simon Cole of the Royal Botanic Gardens, Kew helped me enormously by giving me permission to use their grounds. I am also grateful to the Oxfordshire pig farmer (who wishes to remain anonymous) who was able to supply me with still-born piglets for the UK work. Warm thanks are also extended to Colonel David Whitaker (my father!), who allowed me to use his beautiful garden in Hampshire for the puparia study.

I never imagined when I started this PhD that I would end up carrying out the majority of my fieldwork at the University of Tennessee, Knoxville, USA. During my PhD I have made five visits to the Anthropological Research Facility – four to carry out fieldwork and one to help with the FBI training. The people who work there in the Department of

Anthropology could not have been more accommodating and more helpful. So special thanks must go to: Rebecca Wilson-Taylor, who makes everything happen; Lee Meadows Jantz who runs the Donor Program: Richard Jantz, recently retired Director of the Centre; Murray Marks, who allowed me to help out on the FBI training; and Walter Klippel and Jennifer Synstelien for sourcing and arranging the pigs, which reminds me to also thank the Tennessee pig farmers. A special mention should also go to Professor Bill Bass, who had the vision and courage to open up the Anthropological Research Facility in 1980. I am enormously grateful to the donors themselves and to their families and friends, for being such selfless human beings.

My introduction to the UTK Anthropology Department and the Facility was made possible by Professor Ian Dadour, Director of the Centre for Forensic Science at the University of Western Australia, at the first conference I attended. It is impossible to express the positive impact he has had on my research, and I hope to collaborate with him on future research and publications.

Thanks also to the BBC for facilitating my 2009 trip to Tennessee during the filming of Museum of Life. It was hard work, but enormously enjoyable to work with Ian Hunt (Producer/Director), Tammy Jones (Assistant Producer), James Baker (sound guy) and Chris van Tulleken (presenter and enthusiastic helper).

There are many friends I have to thank, but first a special mention must go to Dr Andrew Hart, forensic scientist at the FSS and Metropolitan Police. His enthusiasm for forensic entomology is infectious and without him, I would not have achieved half as much as I have. We have worked together in Tennessee, collaborated on casework and co-authored papers. I'm sure we will continue to work together in the future.

The Natural History Museum is a fascinating place to work, and there are many people there who deserve my thanks for their friendship and support over the past few years: John Maxen for sorting out administrative issues; Zoe Adams for help and advice in the lab; Nigel Wyatt, whose knowledge on Diptera is infinite; Alfried Vogler and Donald Quicke for their friendship and support.

Finally, I'd like to thank the most important people in my life, for all their love and support over the many years that I have been a student – my best friend, Dawn; my father and late mother; my siblings, Juju and Giles. Last but not least, much love and thanks to Ian "Spanner" Slater, whose patience, kindness and love are inestimable.

CHAPTER 1

INTRODUCTION

1.1 Current knowledge

Forensic entomology is defined as the study of insects and other arthropods in a legal context (Hall, 2001), which can be broadly divided into three main areas (Lord & Stevenson, 1986): urban entomology, e.g. civil actions relating to insects and human environments; stored product entomology, e.g. civil actions relating to insects found in food products; medico-legal entomology, e.g. criminal proceedings in cases of violent crime or unexpected death. The latter, sometimes also referred to as medico-criminal entomology (Hall, 1990) is the most high profile area of forensic entomology, which can be utilised in many ways: neglect of the elderly (Benecke & Lessig, 2001), child abuse (Pickles *et al*., 2005), wildlife poaching (Samuel, 1988; Anderson, 1999), movement of vehicles and transport of human remains (Smith,1986), detection of gunshot residue (Roeterdink *et al*., 2004); detection of drugs, such as paracetamol (O'Brien & Turner, 2004), cocaine (Goff *et al*., 1989; Nolte *et al*., 1992), barbiturates and analgesics (Sadler *et al*., 1995; 1997), opiates, cocaine and barbiturates and some antidepressants (Campobasso *et al*., 2004); heroin (Goff *et al*., 1991), morphine (Hedouin *et al*., 1999a; 1999b); cannabis (Crosby *et al*., 1986). But the most frequent use of forensic entomology is in the estimation of time since death (Ames & Turner, 2003; Hart *et al*., 2008).

Also referred to as post-mortem interval (PMI), the estimation of time since death is usually the most crucial question in cases of untimely death (Wells & Lamotte, 2001), and therefore the most frequent reason for employing a forensic entomologist. Although more traditional methods are used for establishing time since death (Easton & Smith, 1970), such as chemical, histological and bacteriological, these techniques are not reliable because of the variability of post-mortem changes, the lack of scientific investigation, and the

reliance upon subjective evaluation rather than objective measurement (Henssge *et al*., 1988). In addition, most of the post-mortem changes measured by pathologists (e.g. rigor mortis, algor mortis, livor mortis) occur within the first 72 hours of death, so the medical examiner's estimate is limited to these first three days (Dix & Graham, 2000). Despite there being a number of available methods used by pathologists, there is still no single reliable and accurate means of estimating the time since death during the early post-mortem interval (Swift, 2010).

When a person or animal dies, the body starts to decompose almost immediately (Vass *et al.*, 1992). There are many factors affecting the decomposition process, the most important being temperature and humidity, and the environment surrounding the body. However, the body goes through a series of distinct changes, both physical and chemical, from a fresh corpse through to skeletal remains. Although the decay process is continuous, the different stages of decay have been described in discrete terms. Megnin (1894) described eight different stages of decomposition which included the production of odours and fluids as well as some of the physical changes in the body. Payne (1965) listed six stages of decomposition when describing pig cadavers used in his experiments: fresh, bloated, active decay, advanced decay, dry and remains. Rodriguez & Bass (1983) simplified this to four stages of decomposition: fresh, bloated, decay and dry. Anderson & VanLaerhoven (1996) described five stages of decomposition, which have been adopted for use in these studies:

a) **Fresh:** This stage begins immediately after death and continues until the body begins to bloat and/or visibly decompose. During this stage, algor mortis (cooling of the body), livor mortis (pooling of the blood) and rigor mortis (stiffening of the muscles) may occur.

b) **Bloat:** This stage is caused by the rapid proliferation of anaerobic bacteria in the gut due to the breakdown of the immune system, which

results in the excess production of gases, causing a severe swelling of the abdomen. In addition, the scrotum in males can also become excessively swollen, and limbs may become raised off the ground.

c) **Active decay:** This stage is dominated by the feeding of blowfly larvae on the cadaver, resulting in excess liquids and the rapid removal of flesh. The escape of gases from the body generally reduces the effects of bloat.

d) **Advanced decay:** This stage may still have blowfly larvae present, but most of the flesh will have been removed from the body, and cadaverous beetles will be feeding on the dryer tissues such as the skin and hair.

e) **Dry/skeletal:** This stage may last many weeks, months or years, even in warm climates. Any remaining skin will become mummified, and the skeleton will be exposed.

The description of these decomposition stages based on physical appearance alone is, however, very subjective (Early & Goff, 1986) as there is no clearly defined beginning or end to each stage, especially during the dry stage (Rodriguez & Bass, 1983).

In addition to describing eight stages of decomposition, Megnin (1894) also recognised that different species of insect and arthropod are attracted to a cadaver in eight “waves” i.e. at each of the different decomposition stages. Like the decomposition stages, the assemblages of insects attracted at each stage are not distinct, although Rodriguez & Bass (1983) found a direct correlation between the rate of decay and the succession of insects. Smith (1986) also recognised four ecological categories of invertebrates occurring on carrion:

a) **Necrophagous species:** Feed on the carrion itself, and include Calliphoridae (blowflies), Silphidae and Dermestidae (beetles).

b) **Predators and parasites:** Feed on the necrophagous species, and include Silphidae and Staphylinidae (beetles), Muscidae (house flies) and some Calliphoridae eg. *Chrysomya*.

c) **Omnivorous species:** Feed on both the carrion and on the carrion-feeders, and may include Hymenoptera (wasps, bees and ants) and some Coleoptera (beetles).

d) **Adventive species:** Use the cadaver as an extension of their environment, eg. Arachnida (spiders) and Collembola (springtails).

Although many different species of insect are attracted to a decomposing body, the most important insects for the purposes of calculating a post-mortem interval (PMI) are the blowflies (Diptera: Calliphoridae), because they are ubiquitous and usually the first species to be attracted to the body (Greenberg, 1991). The first generation of blowflies, therefore, provides a biological clock that more precisely measures the time since death for two or more weeks than other more traditional pathology-based methods. If the discovery of a body is delayed beyond this, the succeeding colonisers will still provide a useful but less accurate time of death (Greenberg & Kunich, 2002).

Calculation of the age of the first blowfly colonisers therefore provides the most accurate indication of PMI, but it is minimum PMI which is being estimated, because there may be a delay after death (Villet *et al.*, 2010), anything from a few minutes (Anderson and VanLaerhoven, 1996) to many hours, before blowflies oviposit on the body. Oviposition by female blowflies usually occurs in the main facial orifices of the cadaver: the eyes, ears, nose and mouth, preventing desiccation and reducing the chance of predation (Archer & Edgar, 2003). If there are any open wounds on the body, e.g. caused by a stabbing or gunshot, these may also be attractive sites for oviposition (Smith, 1986). These sites of oviposition give easy access to the interior of the body, as newly hatched larvae cannot penetrate normally unbroken skin (Byrd & Castner, 2001). In addition, blowfly eggs may also be laid in the genital area, in the hair,

in any folds of clothes, and also between the body and substrate on which it rests.

There may, however, be a delay in oviposition under certain circumstances. Blowfly activity is significantly reduced at night-time (Greenberg & Kunich, 2002), so if a person dies or a body is deposited or exposed in the evening, oviposition may not occur until the following morning at the earliest. If the body is physically less accessible to blowflies, such as being buried, covered, wrapped, immersed or enclosed, this will also cause a delay in oviposition. Bodies which are found indoors may also be inaccessible to blowflies, or at least delay oviposition, if the windows are shut or if the body is in a high-rise apartment. In studies on piglets in Germany, there was a delay of 24 hours in oviposition on indoor cadavers (Reibe & Madea, 2010). The assemblage of insects found on indoor and outdoor bodies may also vary. Goff (1991) compared the insects found on bodies in 25 forensic cases undertaken in Hawaii, 14 from indoors and 21 from outdoors. Out of a total of 22 species of insect, only five were recovered from both indoor and outdoor bodies. In addition, the indoor bodies had a greater variety of Diptera larvae associated with them, while the outdoor bodies had a greater variety of Coleoptera species present. In a study on 150 interred bodies, many insect orders were recognised, but no Diptera were identified as Calliphoridae (Motter, 1898).

After rate of oviposition and accessibility of the body, the most important factor affecting the rate of blowfly development is temperature: the warmer it is the faster they will develop, conversely the colder it is the slower they will develop (Ames & Turner, 2003). Therefore ambient temperature must always be considered when estimating the age of blowflies and the minimum PMI. In addition, when blowfly larvae reach the later stage of the 2nd instar, and throughout the 3rd instar, they congregate into larval masses. The increased metabolic activity causes an increase in local temperature which results in the larvae within the mass developing at a faster rate than if they were developing at the ambient temperature (Goodbrod & Goff, 1990). Therefore estimations of PMI must be based on the larval mass temperature rather than the ambient

temperature if larvae are, or have been, in the late 2nd or 3rd instar stage, otherwise one is likely to give an under-estimation of the age of the larvae (Turner & Howard, 1992) and thus an overestimation of PMI (Slone and Gruner, 2007). Furthermore, the increase in larval mass temperature remains independent of the ambient temperature (Cianci & Sheldon, 1990), which may be well above the ambient temperature (Ives, 1991). Larvae, however, have a lethal maximum temperature limit, above which they will die. Therefore the difference between the larval mass temperature and the ambient temperature is likely to be greater in cooler ambient temperatures (Doenier, 1940; Joy *et al*., 2002). In addition, the increased temperatures of the larval mass may also be influenced by the number of larvae in the mass (Marchenko, 2001) and by the site and dimensions of the aggregation (Turner and Howard, 1992).

In calculating PMI, the lower development temperature (LDT) for each species must be considered, as these may vary for different species, and with geographic location (Gennard, 2007). For example, three different base temperatures have been suggested for the common UK blowfly species, *Calliphora vicina*: 3.5ºC in the North of England (Davies & Ratcliffe, 1994), 2ºC in Russia (Marchenko, 2001) and 1ºC in London (Donovan *et al*., 2006).

When fly larvae have finished feeding towards the end of the 3rd instar stage, they move off the body to pupate. Although they can still be utilised to estimate post-mortem interval, if a previous wave of larvae which have already left the body to pupate are overlooked, the PMI will be underestimated (Greenberg, 1990). Different species have been found to move varying distances, with some species staying close to the carcass to pupate, whilst others move some distance away (Greenberg, 1990). In addition, different substrates may be a strong influencing factor, as the same species have been found to move from 12-15ft from the carcass (Cragg, 1955), and from 80-100ft on hard ground (Green, 1951). Laboratory studies have been carried out in this area, but they are constrained to working within certain space limits, such as rectangular troughs measuring 8.1m x 22cm (Greenberg, 1990), 3m x 30cm (Godoy *et al*., 1995, 1996), and a circular arena measuring 50cm in diameter (Gomes & von Zuben, 2005).

Once the larvae have pupated, and the adult flies have emerged, only the empty pupal cases remain. If a body remains undiscovered for some time, the empty pupal cases may be the only insect evidence available for analysis and estimation of post-mortem interval. In many cases this may have limited value, as the only sure conclusion can be that flies living on the body have been through one complete generation. In some cases, however, the important question may be whether or not the body decomposed in a particular place, and the presence of a significant number of pupal cases may be a good indicator of this. Also, some seasonal information may be gleaned from the presence of empty puparia, as different insects occur at different times of the year, and bodies exposed in the spring and summer seasons will have a richer fauna than those exposed in the winter (Smith, 1986). Little published information exists, however, on how long pupal cases remain in the environment before they disintegrate or decompose, and these tend to be either reported from archaeological sites (Gilbert & Bass, 1967; Stafford, 1971; Teskey & Turnbull, 1979; Faulkner, 1986) or from casework, such as Nuorteva (1987) who reported empty pupal cases of *Phormia terraenovae* Robineau-Desvoidy, 1830 being found four years after a body decomposed.

When carrying out forensic casework and analysis, the entomologist must be familiar with the local fauna, and able to identify the specimens to species level, whether they are in the immature or adult stage. If the species is incorrectly identified, the analysis will most likely be inaccurate, as even closely related species may have different rates of development. Field studies can be carried out in order to determine the local entomofauna, such as those in the United States (Payne, 1965; Payne *et al*., 1968a, 1968b; Payne & King, 1969, 1970, 1972).

The most common species of blowfly found in the UK are *Calliphora vicina* Robineau-Desvoidy, 1830 which is present throughout the year, together with *C. vomitoria* (Linnaeus, 1758), *Lucilia sericata* (Meigen, 1826), *L. illustris*

Meigen, 1826 and *L. caesar* (Linnaeus, 1758), all of which are present from early spring to late autumn. A number of studies have been carried out using laboratory-bred species, in order to ascertain developmental rates to enable estimations of PMI to be made: *C. vicina* from the UK, over a range of temperatures (Donovan *et al*., 2006); *C. vicina* and *C. vomitoria* at 5ºC (Ames & Turner, 2003); *C. vicina* in Austria (Reiter, 1984); *C. vicina* at 3ºC (Johl & Anderson, 1996); twelve species, including *C. vicina, C. vomitoria* and *L. sericata* in the US (Kamal, 1958); *C. vicina*, *C. alpina* (Zetterstedt, 1845), *C. vomitoria*, *Phormia terraenovae* and *L. sericata* at 3.5-26ºC (Davies & Ratcliffe, 1994); five species in the US, including *C. vicina*, *C. vomitoria* and *L. sericata* (Greenberg, 1991); *C. vomitoria* in the US (Greenberg & Tantawi, 1993); *L. sericata* in the US (Ash & Greenberg, 1975), in the UK (Wall *et al*., 1992) and in Austria (Grassberger & Reiter, 2001); *Musca domestica* Linneas, 1758 at 5ºC (Leopold, 2000); *Protophormia terraenovae* at 4ºC (Myskowiak & Doums, 2002).

There are a number of problems with using these data to estimate PMI in forensic cases occurring in the UK. Firstly, many of these studies are carried out using species of fly which do not occur in the UK, so their developmental rates may be different from those species which commonly occur in the UK. Even where the same species are also found in the UK, different populations outside of the UK may have different developmental rates.

Laboratory studies are usually carried out under controlled conditions, excluding seasonal effects, such as wind, rain and sunshine. The environmental and breeding conditions are closely regulated and monitored, namely temperature, humidity, lighting, population size and food resources. The effect of temperature is usually the main reason behind the experiment, so it is generally fixed. Humidity in the UK is not extreme, and therefore this is generally monitored, but may not be regulated, and lighting conditions are generally fixed at 12H light, 12H dark. The food type provided for the larvae is typically liver-based and of animal origin, although studies on *C. vicina* (Kaneshrajah &

Turner, 2004) and *L. sericata* (Clark *et al*., 2005) have shown that their growth rates vary depending on which body tissues they are fed on.

Population sizes for breeding stock, and perhaps experimental studies, are usually limited to the size of breeding cage being utilised. The amount of food provided is also regulated according to population size in order to reduce the negative effects of intraspecies competition which may occur naturally when the insect populations are high and the food resource is limited, resulting in the development of smaller larvae and adult flies. Laboratory studies also prohibit interspecies competition, unless deliberately introduced (Goodbrod & Goff, 1990), which may occur due to the natural succession of insects on a cadaver, either between closely related blowfly species, or by beetle larvae and adults predating on blowfly larvae, which can severely reduce their numbers.

Succession studies carried out in the field have used a large array of animal species, including snails (Beaver, 1972, 1977), toads and lizards (Cornaby, 1974), gulls (Lord & Burger, 1984a), chickens (Hall & Doisy, 1993), seals (Lord & Burger, 1984b), racoons (Joy *et al*., 2002), rats (Utsumi *et al*., 1958; Keiper *et al*., 1997), mice (Isiche *et al*., 1992), mice and quail (Smith & Wall, 1997), voles (Lane, 1965), rabbits (Tantawi *et al*., 1998), dogs (Reed, 1958), foxes (Easton, 1966; Smith, 1975), cow liver (Hanski, 1976; Hanski & Kuusela, 1977), pig liver (Hwang & Turner, 2005), impala (Ellison, 1990), moose (Samuel, 1988), monkeys (Omar *et al*., 1994) and elephants (Coe, 1978). Kneidel (1984) used a range of 22 species of animal cadaver to determine how Diptera use alternative carcass types: slugs (two species), snails (three species), millipedes, beetles (two species), salamanders (four species), frogs, toads (two species), snakes (four species), mice, rats and shrews; he also reports having reared Diptera from a brown-headed cowbird and a box turtle. The results suggested that Diptera do not breed indiscriminately, but rather choose the type of taxon, and that small mammals were inhabited by one of two particular species of Diptera, *Phaenicia caeruleiviridis* Macquart, 1855 or *C. vicina* depending on the season, whereas arthropods and reptiles were inhabited by Diptera species with weaker

seasonal preferences but with distinct preferences for certain types of carrion. Kneidel (1984) concluded, therefore, that mammal carcasses are large enough to decompose relatively slowly, thus accommodating a succession of Dipteran species, whereas small invertebrates and reptiles decompose so quickly that only the primary species is able to inhabit them. A study conducted on two different sizes of pig concluded that there was no difference in composition or successional order of arthropod species, but that the larger carcass attracted a greater number of arthropods and decomposed faster than the small carcass (Hewadikaram & Goff, 1991).

The most widely accepted model for forensic entomology studies, however, is the domestic pig, *Sus scrofa domestica* Linnaeus, 1758 (Payne, 1965; Payne *et al*., 1968a, 1968b; Payne & King, 1969, 1970, 1972), for economic, ethical and biological reasons. Economically, pig carcasses can be readily acquired more cheaply than many other animal carcasses, especially primates. Ethically, pigs are already bred in large numbers for the food industry, and are therefore more socially acceptable as a tool for scientific research. Biologically, pigs have more anatomical similarities to humans than do most other animals (Clark *et al.*, 2005), in many aspects of both infant and adult anatomy, physiology, biochemistry, pathology and pharmacology, in addition to being virtually hairless and with abdominal skin very similar to that of humans in texture, permeability and thickness (Sullivan *et al.*, 2001), therefore pigs are the animal generally used in decomposition studies (Goff, 2000).

The research work on pigs is valuable in terms of succession studies, but when used in criminal investigations, the validity of extrapolating from these studies to human corpses has been questioned (Catts & Goff, 1992). To date, just one published study has been conducted to assess the suitability of using pigs as surrogate humans in the context of forensic entomology. Two pigs and one human cadaver were decomposed at the Anthropological Research Facility at the University of Tennessee, Knoxville, and the succession of insects on each was recorded (Haskell, 2000; Schoenly *et al*., 2007). They concluded that the composition and successional order of arthropods overlapped sufficiently to

recommend the use of pig cadavers as a substitute for human cadavers in forensic entomology research and training. Their primary reservation of the results was the lack of replication possible due to the limited availability at the time of suitable human cadavers. In addition, they only compared the faunal composition on both species of cadaver, i.e. the succession of insects. However, no comparison of blowfly development was made between the two types of cadaver, and therefore no conclusions regarding PMI could be drawn. Clark et al (2005) stated that larvae grow more rapidly on pig than on cow, and therefore the possibility that there are differences in growth rate and size of larvae needs to be examined between humans and pigs.

1.2 Aims and objectives

Forensic entomology is defined as the study of insects and other arthropods in a legal context. Therefore much of the research that is carried out aims to investigate and answer questions which arise in carrying out forensic entomology casework. The research presented in this thesis aims to address some areas which are less well-studied, or where novel techniques can be utilised.

Chapter Two investigates the dynamics of larval masses of the UK's most common species of blowfly, *Calliphora vicina*, by breeding larvae at different densities in order to determine the minimum number of larvae in a larval mass which will result in an increase in the larval mass temperature above the ambient temperature. Where there is a high larval load on a cadaver, the food resource may be limited, and the effect of competition on development is also considered as it may result in smaller individuals which, if measured for a minimum post-mortem analysis, could result in the underestimation of time since death.

There are a number of different ways in which the temperature of larval masses can be measured. A standard medical thermometer is cheap and easy to obtain, but as there is a delay for the temperature to register and it can be

difficult to read, the reading may not be accurate. In addition, inserting an object such as a thermometer directly into a larval mass disturbs the larvae, causing them to disperse, which results in a cooling of the larval mass temperature and, clearly, disruption of subsequent normal activity of larvae within the mass. This problem also occurs when using a digital probe, although the temperature is quickly and accurately displayed and it should, therefore, be used in preference to a mercury-based thermometer. An infrared thermometer also gives a quick and accurate temperature reading and one major benefit of using this method is that it is non-invasive, the temperature being measured from a distance of about 15 cm, so that the larval mass is not disturbed and a more consistent temperature reading over time is given. The limitation of these three methods is that the temperature of only a small area of the larval mass is being measured at any one time. In order to measure a range of temperatures from a single larval mass, the infrared thermometer or probe has to be moved around or, in the case of the probes, reinserted a number of times, thus disturbing the larval mass even more. Therefore when carrying out controlled experiments on cadavers, electronic Tinytag® dataloggers are used with probes attached which can be inserted directly into the body, usually the mouth and rectum, at the start of the experiment. These dataloggers can be programmed to record the temperature at set intervals, and for an extended period of time. The use of probe dataloggers for measuring internal body temperatures would not be permissible when carrying out forensic casework, however, as they are highly invasive and could cause post-mortem wounds to the body. The “button” datalogger works in the same way, and because it can be completely hidden inside the body it is useful if, for instance, the cadaver is being filmed and the visual impact of trailing wires needs to be minimised. However, removing it at the end of the experiment can be difficult due to its small size.

One novel method of measuring and recording the temperature of larval masses is the use of infrared thermal imaging, and the technique was used in this research project in a number of experiments. Firstly, thermal imaging was used to measure the fall in the temperature of individual post-feeding larvae, when they were removed from an incubator at high temperature to a lower

ambient room temperature. Thermal imaging was then utilised on a larval-infested piglet cadaver, demonstrating the ability to measure the surface temperature of an entire body over time, non-invasively. Finally, thermal imaging was used on human cadavers in Tennessee, to assess the practicality of recording temperatures using this method, versus the more traditional method of probes and infrared datalogger. The method was also used to ascertain whether there is a correlation between the size of larval masses and the temperatures generated, and also to investigate heat retention and heat loss in larval masses.

In forensic casework, bodies may be discovered indoors or outdoors, but limited research has been carried out to assess what effect this may have on larval development and minimum PMI estimates. In Chapter Three, this subject is investigated by a five-replicate study whereby piglet cadavers were placed both indoors and outdoors, over different seasons. Although one would expect the ambient temperature and the rate of decomposition of the piglets to vary depending on season, the aim of the experiment was to investigate whether oviposition rates differ between indoor and outdoor cadavers, and whether the resulting estimated post-mortem intervals differ. Most studies of blowfly larval development are carried out in the laboratory, so a further study was carried out on piglets in a natural environment, to assess whether development rates recorded in the field corresponded to published development rates generated by laboratory studies.

Forensic entomology is most often utilised in crime scene investigation when the methods used by pathologists to estimate PMI are no longer of use, i.e. after 48 hours or so. Often when a body is discovered at a crime scene or deposition site, the blowfly larvae have finished feeding on the body and have moved off the body to pupate. In some cases, the first generation of flies have completed their development and emerged from their puparia, leaving just the empty puparial cases. In these circumstances, some temporal information can still be gleaned from the presence of empty puparia, such as a minimum post-mortem interval, whether the body decomposed in that place, and seasonal data, based on the fly species which emerged from the puparia. However, no

research has yet been undertaken to ascertain the length of time that empty puparia remain sufficiently intact to be recoverable for analysis. Chapter Three of this work presents the first long-term study, carried out over six years, to assess this issue, with the intention of providing some indication of for how long after death forensic entomology analysis can provide information of value to the criminal investigation.

Although extensive research has been carried out on animal models, mainly pig cadavers, most forensic entomology casework involves the recovery of insect evidence from human cadavers, the exception being cases of wildlife crime or neglect of domestic pets or farm animals. Forensic entomology casework involving human cadavers is fairly sporadic for most forensic entomologists and even if being regularly called upon to attend crime scenes and post-mortems, the opportunity for working with human cadavers is fairly limited. In addition, one is, of course, restricted to collecting evidence off the body when it is at a single stage of decomposition. It is not possible to examine the body and the insects on it prior to the discovery of the body, nor is it possible, for ethical reasons, to continue to study the body as it decomposes. Therefore the opportunity to study the insect activity and the decomposition process over a period of time on a human body is invaluable, as outlined in Chapter Four. Although only one exposed cadaver was studied at this time, valuable insights into field methodologies, insect behaviour and ecology were gained, which were applied in subsequent chapters and led onto further research. During study of the exposed cadaver, a second cadaver was exposed in a situation that provided much reduced accessibility to blowflies, i.e. it was enclosed in a plastic “wheelie” bin. This practical study was carried out as a comparison to previous studies conducted by a co-researcher on pig cadavers in cars, “wheelie” bins being a body disposal tool used by criminals in the UK and overseas. Although there were no replicates the hypothesis being tested was that the insect faunal succession on the body in the bin would be interrupted due to the relative inaccessibility to blowflies.

Forensic entomology research on blowfly development and behaviour is generally concentrated on the larval feeding stage. However, an understanding

of the post-feeding dispersal behaviour of Calliphorids is important to the practising forensic entomologist, because the oldest insects may have left the body to pupate, and therefore sampling only the larvae present on the body could lead to an underestimate of the PMI. Casework at the NHM has included examples where police SOCOs have not collected either dispersing larvae or pupae and so have provided evidence of suboptimal quality. Opportunities to study the dispersal behaviour in a natural environment are rare, because it occurs over a relatively short period of time and is therefore seldom witnessed during the course of police investigations. Controlled studies are difficult because there are so many factors involved, and because so little is understood about larval migration, such as the distance over which larvae will travel before pupating. Working in Tennessee allowed the opportunity to observe the dispersal of blowfly larvae from a number of cadavers, and to investigate a number of common assumptions.

Most forensic entomology research is carried out using pigs as a model for humans. But the question of whether pig cadavers are a good representation of human cadavers, with respect to insect development, especially blowflies, has largely been overlooked. Chapter Five seeks to address this assumption by carrying out a six-replicate study on pigs and humans. The hypothesis was that no significant differences would be found between blowfly behaviour and development on both types of cadaver, with respect to succession of insects, blowfly species attracted, oviposition sites, larval development, larval masses and post-feeding larval dispersal. Positive results in support of the hypothesis will enable practising forensic entomologists to present their findings in court with confidence.

CHAPTER 2

LARVAL MASSES

2.1 Introduction

The most important factor affecting the rate of blowfly development is temperature, resulting in more rapid development in warmer temperatures and slower development in colder temperatures (Ames & Turner, 2003). Therefore the ambient temperature, defined by season and/or environment, will usually determine the rate at which blowflies develop. When, however, Calliphorid larvae reach the late 2nd instar, and throughout the 3rd instar, they congregate and feed in larval masses. The increased friction and combined metabolic and enzyme activity results in an increase in local temperature which has been recorded well above the ambient temperature (Doenier, 1940; Joy *et al*., 2002), thereby increasing the development rate of the larvae.

No studies to date have been carried out to determine the precise number of larvae in a larval mass which is required to raise the local temperature above ambient, although a study of larval masses on pig carcasses found a direct correlation between the volume and core temperature of larval mass (Slone & Gruner, 2007). A forensic entomologist attending a scene will generally measure the temperature of any larval masses prior to collecting samples. But larval masses are easily disturbed due to increased human activity and the removal of coverings such as clothes, and even the removal of the body to the mortuary. In these circumstances it may be difficult to determine the temperature of any larval masses, i.e. the temperature at which the larvae were developing.

Laboratory studies designed to determine the development rate of specific species of blowfly are generally carried out using small numbers of larvae (e.g. Donovan *et al*., 2006) in order to reduce possible effects of larval masses i.e. elevated temperatures. In addition, these studies are generally carried out

using incubators which have been set to a specific temperature. This means that any elevated larval temperatures will be counteracted by the constant regulation of the incubator. Furthermore, laboratory studies will usually ensure a sufficient food resource for the number of larvae, whereas competition for food may affect both larval development rate and adult size (Ireland & Turner, 2006). Lane (1975) suggested that competition is more critical to development in the larval stage than in the adult stage, and that food and space are the primary resources competed for during the larval stage. Saunders & Bee (1995) concluded that approx. 1g of food is adequate food for one larva of *Calliphora vicina*.

When collecting samples of larvae, either during casework or for research purposes, the larval mass temperature is usually recorded, and a variety of equipment is available for this purpose (Hall, Whitaker & Richards, 2012). During research, electronic Tinytag® probes may be used, which are permanently inserted into the body cavities during the duration of the experiment. The benefit of these is that continual recording of the internal body temperatures of the cadaver is possible, at preset time intervals, without disturbing the feeding larvae or breaking up the larval masses. When undertaking forensic casework, however, it is not possible to insert probes into the body or to record temperatures over a long period of time, so temperatures are recorded on a one-off basis just prior to sampling. Many researchers and case workers use either a standard medical thermometer, or a probe attached to a digital recorder, both of which are invasive and result in disturbance of the larval mass being recorded (Hall, Whitaker & Richards, 2012). To avoid breaking up the larval mass, an infra-red thermometer can be used instead, which although not invasive, only records a small area (i.e. a few mm^2) of the surface temperature of the larval mass. The continual churning of the larvae while feeding means that the temperature at any single point is constantly fluctuating, and a larval mass of any significant size may be recorded as having a range of temperatures if a number of recordings are taken in succession. Aside from electronic dataloggers, another limitation of these commonly-used thermometers is that the temperature must be written down by hand, before being manually entered into a spreadsheet for analysis.

In order to record continuous temperatures across an entire larval mass or even an entire body, a thermal imaging camera can be utilised. These can record both still images and video, and they are sensitive enough to record the temperature of individual larvae, which enables highly accurate analysis of the dynamics of larval masses. In addition, the captured images are automatically recorded and can be analysed in detail.

2.2 Larval development

2.2.1 Aims

The aim was to produce *in vitro* larval masses of differing densities in order to determine the minimum number of blowfly larvae in a larval mass which is able to generate an increased local temperature above the ambient temperature. The hypothesis being tested was that even small numbers of larvae would generate a local temperature higher than the ambient temperature, but that larger cultures of larvae, generating a higher local temperature, would develop faster than smaller cultures of larvae. In addition, the ratio of food resource to the number of larvae was also considered in order to counteract any effects of competition on larval development. The hypothesis being tested was that competition for food resources would result in smaller individuals in the pupal and adult stage.

2.2.2 Materials and methods

2.2.2.1 Experiment 1

On Day 1, ~25g of fresh lamb's liver was placed into a breeding cage holding approximately 50 individuals, male and female, of *Calliphora vicina* (Diptera: Calliphoridae), which were 11th generation from wild stock caught in Central London. On Day 2, the eggs which had been laid were removed from the liver, rinsed in distilled water, and placed on damp filter paper in a Petri dish and

covered with gauze. The ambient room temperature at this time was 16ºC. By the morning of Day 3 the eggs had not yet hatched so the Petri dish was moved to a warmer location with an ambient temperature of 20ºC, where they started hatching later that day between 5:30pm and 9:30pm. The Petri dish was loosely wrapped in a plastic bag and left overnight. On Day 4, the 1st instar larvae were removed individually using a fine paint brush and placed on lamb's liver in 23cl polystyrene cups. The cultures were set up with 1st instar larvae rather than eggs, to ensure an accurate predetermined number of larvae were used, as eggs may not be 100% viable. Three replicates of four cultures were set up with different quantities of liver and different numbers of larvae (Table 2.2.2.1).

Table 2.2.2.1 Experiment 1: Four cultures of *Calliphora vicina* 1st instar larvae

Culture	**Quantity liver**	**No. of larvae**	**Ratio grams liver : no. larvae**
A	20g	20	1:1
B	20g	200	1:10
C	200g	200	1:1
D	100g	400	1:4

A minimum ratio of 1g liver to 1 larva (1:1) was used in two (cultures A and C) of the four cultures, being the minimum required to reduce crowding and enable full development of the larvae (Saunders & Bee, 1995). Two further ratios were also used in order to a) assess this assumption (culture B – 1:10), and b) increase the numbers of larvae without using an excessive amount of liver (culture D – 1:4).

The larvae were kept in a room with naturally fluctuating temperatures, buffered from external temperatures, ranging from 13.5°C-18.4°C (mean=15.9°C). Once the 1st instar larvae were placed on the liver, the ambient and larval mass temperatures were measured using a digital probe thermometer twice a day, morning and afternoon, over the following seven days, and the morning of day eight and the afternoon of day nine. The exact intervals were: 19, 22.5, 39.5, 50, 64.5, 69, 88.5, 94.5, 112, 120, 136.5, 142.5, 160.5, 165.5, 184.5 and 201.5 hours.

Once the larvae had reached the 3rd instar (specifically after 142.5 hours), each pot was placed in an individual bowl containing sawdust, into which post-feeding larvae could disperse to enable pupariation. After 185 hours a sample of 3rd instar larvae were killed by immersion in freshly boiled water: 3 larvae from each Culture A and 10 larvae from each Culture B, C and D. The larvae were measured using an eyepiece graticule under a binocular microscope at x6 magnification. Once the remaining larvae had pupated, all pupae from Culture A and 50 randomly selected pupae from Cultures B, C and D were measured as follows: weight, length, width and depth, and the volume was calculated (width x depth x length x π ÷ 6). The pupae were reared to adult flies which were killed by placing them in a freezer. The left wing was then removed from all the Culture A flies and from 20 flies (10 male and 10 female) from each Culture B, C and D. Posterior cross-vein dm-cu was measured using an eyepiece graticule under a binocular microscope at x25 magnification, as a measure of adult size (Ireland & Turner, 2006).

2.2.2.2 Experiment 2

The experiment was repeated using the same protocol described in Section 2.2.2.1, but with the further aims of: a) increasing the number of larvae in some of the cultures, and b) using ratios of grams of liver to number of larvae no greater than 1:4 in order to reduce competition for the food resource. Because larger numbers of larvae were required, only two replicates of four cultures could be set up (Table 2.2.2.2), but this was deemed sufficient to conduct this experiment.

Table 2.2.2.2 Experiment 2: Four cultures of *Calliphora vicina* 1st instar larvae

Culture	**Quantity liver**	**No. of larvae**	**Ratio grams liver : no. larvae**
A	20g	20	1:1
B	10g	100	1:1
C	250g	500	1:2
D	250g	1000	1:4

The required number of 1st instar larvae could not be obtained within a single day therefore the cultures were set up on two separate days: Cultures A1, C1

and D1 were set up at 12:00 midday one day, and the remaining cultures were set up 20 hours later at 08:00 the following day. The larvae were kept in a room with naturally fluctuating temperatures, buffered from external temperatures, ranging from 21.1°C-23.9°C (mean=22.9°C). The ambient and larval mass temperatures were recorded at the following time intervals after the second set of cultures were set up: 6, 18, 6, 17, 6.5, 17, 7, 17, 5, 20, 6.5, 17.5, 5.5, 18.5, 9, 15.5, 5.5, 19, 5 hours. In addition to the measurements taken in Experiment 1, in Experiment 2 another culture was set up, S1-S4, each with 20g liver and 20 larvae (ratio 1:1), from which larvae could be sampled and measured instead of sampling from Culture A. During Experiment 2, larvae were sampled, killed and measured daily from each culture (five larvae from Cultures B and S, and ten larvae from Cultures C and D). The ADH (Accumulated Degree Hours) was also calculated for Experiment 2 using an LDT (lower developmental threshold) of 1°C, where ADH=∑(hourly temp-1).

2.2.3 Results

The results of Experiments 1 and 2 were considered separately due to: the different receptacles used; the different number of replicates of each culture; the different room temperatures between each experiment; the pupal measurements were carried out by two different people, potentially resulting in subjective variation [pupae in Experiment 1 were measured by Claire Foggon, a work experience student from St. Hilda's, Oxford; pupae in Experiment 2 were measured by the author]. However, taking these variations into account, the results of both experiments were also combined.

2.2.3.1 Experiment 1

Figure 2.2.3.1 shows an increase in temperature of all four cultures above the ambient temperature, with the greatest number of larvae, Culture D (100g/400L), exhibiting the highest temperature increase, nearly 5°C above ambient. Culture C (200g/200L) temperatures reached nearly 4°C above ambient. The remaining two cultures had the smallest number of larvae:

Culture B (20g/200L) temperatures reached nearly 3°C above ambient, but Culture A (20g/20L), exhibited the lowest temperature increase, 1°C above ambient. The larvae in Culture A (20g/20L) were the slowest to develop. After 201.5 hours only Culture A had not yet dispersed, and after 271 hours they were still in the post-feeding stages, whereas Cultures B, C and D were all in the pupal stage by that time.

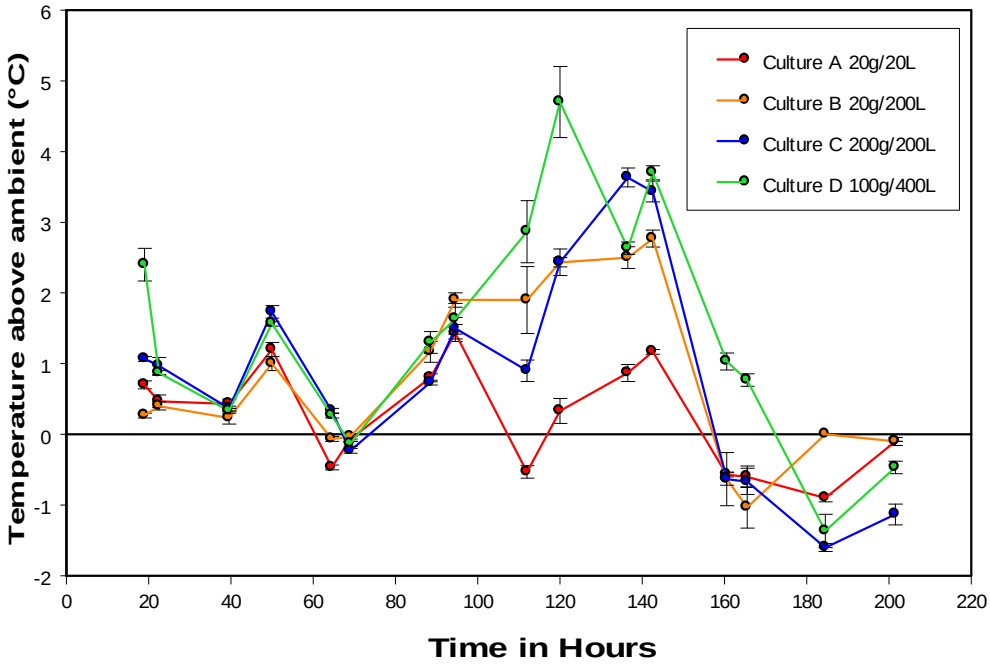


Figure 2.2.3.1 Experiment 1: Temperature difference between larval masses and ambient temperature recorded for each of four cultures of *Calliphora vicina*: Culture A (20g/20L), Culture B (20g/200L), Culture C (200g/200L), Culture D (100g/400L), raised at an ambient temperature ranging from 13.5°C-18.4°C (mean=15.9°C)

Culture B, with the largest larval to food ratio of 1:10 (20g/200L) resulted in the smallest pupae and adults (Figure 2.2.3.2). Culture D with a ratio of 1:4 (100g/400L) had the next smallest pupae and adults. Cultures A (20g/20L) and C (200g/200L), with a ratio of 1:1, resulted in the largest pupae and adults. T-tests carried out on the data show a significant difference between all cultures ($P<0.002$, mostly $P<0.0001$) except between Culture A (20g/20L) and Culture C (100g/400L) ($P>0.43$).

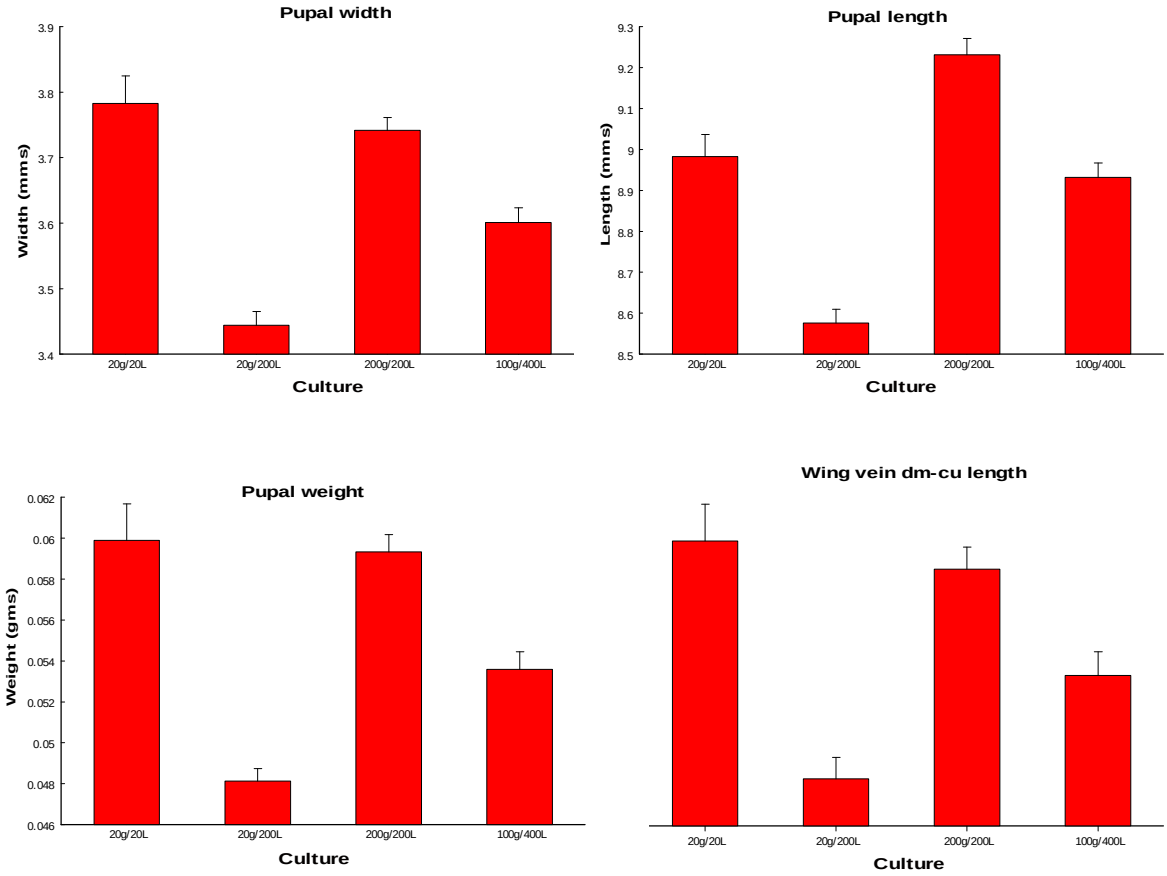


Figure 2.2.3.2 Experiment 1: Pupal width, length and weight, and adult wing vein dm-cu measurements, for each of four cultures of *Calliphora vicina*: Culture A (20g/20L), Culture B (20g/200L), Culture C (200g/200L), Culture D (100g/400L), raised at an ambient temperature ranging from 13.5°C-18.4°C (mean=15.9°C)

Not including sampled larvae, percentage survival and mortality from 1st instar to the pupal stage, and 1st instar to the adult stage, is shown in Table 2.2.3.1. Culture A, with the smallest number of larvae and food resource, exhibited the lowest mortality up to the pupal stage, but the mortality increased thereafter. The other three cultures exhibited similar rates of mortality.

Table 2.2.3.1 Experiment 1: Survival and mortality from 1st instar to pupal stage and 1st instar to adult stage for *Calliphora vicina* larvae reared in four different cultures

Experimental set-up			**1st instar to pupal stage**		**1st instar to adult stage**	
Culture	**Density**	**Ratio**	**% Survival**	**% Mortality**	**% Survival**	**% Mortality**
A	20g/20L	1:1	92.1	7.9	64.7	35.3
B	20g/200L	1:10	80.2	19.8	71.9	28.1
C	200g/200L	1:1	78.8	21.2	72.1	27.9
D	100g/400L	1:4	76.1	23.9	64.2	35.8

Chi-square distribution tests carried out on the survival of 1st instar to pupal stage gave a $p<0.05$ for all cultures, except for B and C ($p=0.4125$) and C and D ($p=0.1424$). Chi-square distribution tests carried out on the survival of 1st instar to adult stage give a $p<0.05$ for two sets of cultures, the remaining giving p values of A and B ($p=0.2509$), A and C ($p=0.2387$), A and D ($p=0.9385$) and B and C ($p=0.9256$).

2.2.3.2 Experiment 2

Figure 2.2.3.3 shows the highest increase in temperature of all four cultures above the ambient temperature, with the greatest number of larvae, Culture D (250g/1000L), exhibiting the highest temperature increase, almost 5°C above ambient. Culture B (100g/100L) temperatures reached nearly 2°C above ambient, Culture C (250g/500L) temperatures reached 1°C above ambient, and Culture A (20g/20L), exhibited the lowest temperature increase, just under 1°C above ambient.

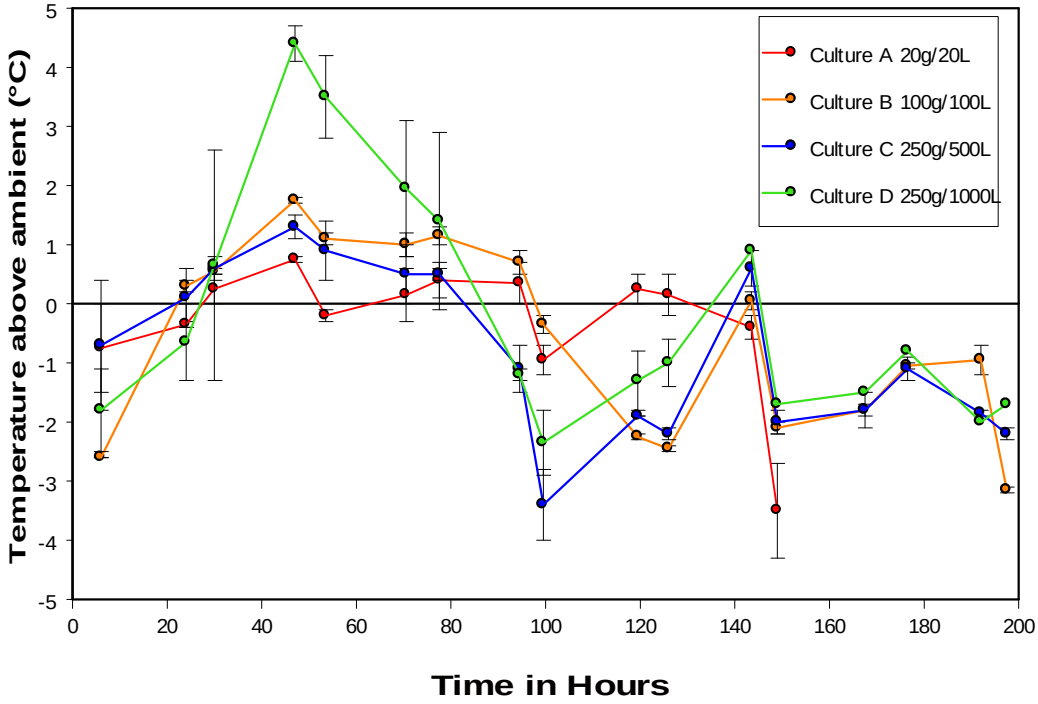


Figure 2.2.3.3 Experiment 2: Temperature difference between larval masses and ambient temperature recorded for each of four cultures of *Calliphora vicina*: Culture A (20g/20L), Culture B (100g/100L), Culture C (250g/500L), Culture D (250g/1000L), raised at an ambient temperature ranging from 21.1°C-23.9°C (mean=22.9°C)

One of the two cultures with a ratio of 1:1 (20g/20L) resulted in the greatest pupal volume and pupal weight, but the other (100g/100L) had a lower mean pupal weight than the other three cultures (Figure 2.2.3.4). The cultures with a ratio of 1:2 (250g/500L) and 1:4 (250g/1000L) gave very similar results. The length of wing vein dm-cu was also smaller in adults with the ratio of 1:1 (100g/100L).

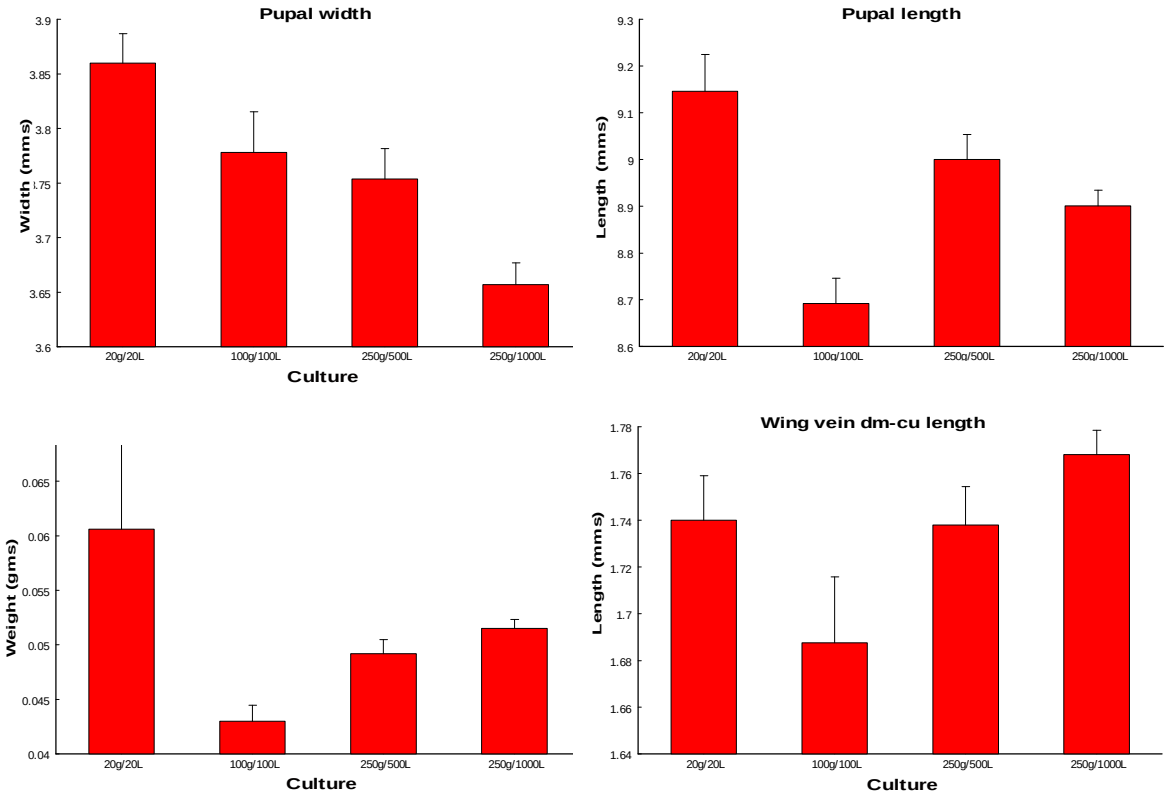


Figure 2.2.3.4 Experiment 2: Pupal width, length and weight, and adult wing vein dm-cu measurements, for each of four cultures of *Calliphora vicina*: Culture A (20g/20L), Culture B (100g/100L), Culture C (250g/500L), Culture D (250g/1000L), raised at an ambient temperature ranging from 21.1°C-23.9°C (mean=22.9°C)

Not including sampled larvae, percentage survival and mortality from 1st instar to the pupal stage, and 1st instar to the adult stage, is shown in Table 2.2.3.2.

Table 2.2.3.2 Experiment 2: Survival and mortality from 1st instar to pupal stage and 1st instar to adult stage for *Calliphora vicina* larvae reared in four different cultures

Experimental set-up			**1st instar to pupal stage**		**1st instar to adult stage**	
Culture	**Density**	**Ratio**	**% Survival**	**% Mortality**	**% Survival**	**% Mortality**
A	20g/20L	1:1	80.0	20.0	40.0	60.0
B	100g/100L	1:1	61.3	38.7	10.6	89.9
C	250g/500L	1:2	47.5	52.5	31.0	69.0
D	250g/1000L	1:4	73.4	26.6	51.9	48.1

Chi-square distribution tests carried out on the survival of 1st instar to pupal stage give a $p<0.05$ for all cultures, except for A and D ($p=0.3486$). Chi-square distribution tests carried out on the survival of 1st instar to adult stage give a $p<0.05$ for all cultures, except for A and C ($p=0.2178$) and A and D ($p=0.1325$).

The ADH (Accumulated Degree Hours) was calculated for the results in Experiment 2 (Figure 2.2.3.5), using a lower developmental threshold of 1°C which was estimated to be suitable for *Calliphora vicina* (Donovan *et al.*, 2006). The larvae reached their maximum length of approximately 18mm after 2000 to 2800 ADH.

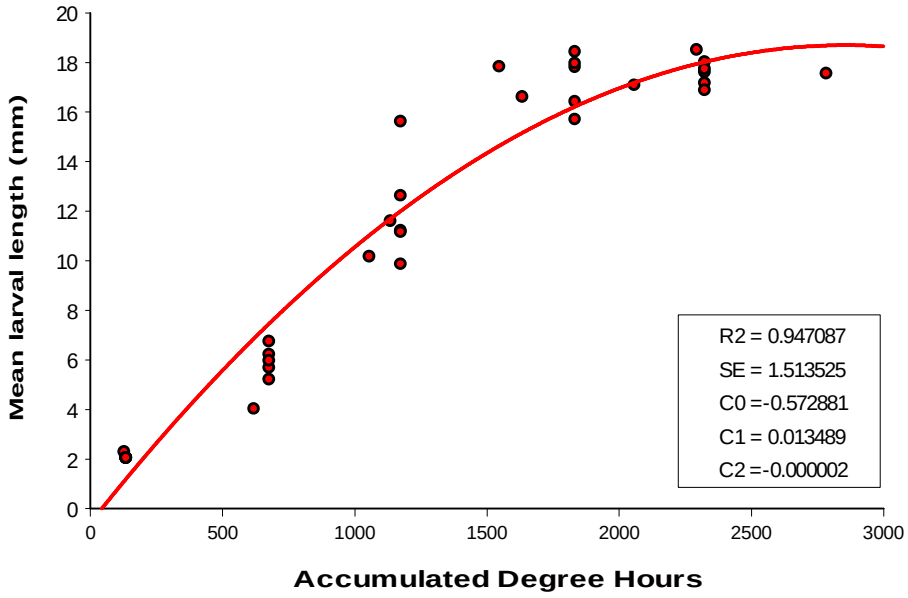


Figure 2.2.3.5 Experiment 2: Accumulated Degree Hours (ADH) calculated for four cultures of *Calliphora vicina*: Culture A (20g/20L), Culture B (100g/100L), Culture C (250g/500L), Culture D (250g/1000L), raised at an ambient temperature ranging from 21.1°C-23.9°C (mean=22.9°C). This is a second order polynomial, formula is $Y = (c2 * x^2) + (c1 * x) + c0$

2.3 Thermal imaging on Calliphorid larvae

2.3.1 Aims

The aim was to monitor the heat retention and heat loss of 3rd instar post-feeding blowfly larvae at different densities using thermal imaging. The expectation was that smaller numbers of larvae would remain at a temperature close to the ambient temperature, whereas larger numbers of larvae would retain a local temperature above the ambient temperature.

2.3.2 Materials and methods

2.3.2.1 Individual larvae

Post-feeding 3rd instar larvae of *Calliphora vomitoria* (Diptera: Calliphoridae) were purchased from a commercial fishing shop and stored in a fridge at 3ºC-4°C. They were counted out into quantities of 1, 5, 10 and 40 larvae in individual 23cl polystyrene cups (Figure 2.3.2.1) and then put into an incubator set to 25ºC. After four hours the cups containing larvae were removed from the incubator into a room with an ambient temperature of 19ºC-21ºC. There were two replicates of the four quantities.

Thermal images were taken using a FLIR Systems AGEMA 570 infrared camera, which has a spectral range of 7.5-13.0 microns, temperature measurement range of -20°C to + 2000°C and temperature measurement accuracy of ±2% or 2° (full specification available from www.flir.com). Thermal images were taken at set intervals, up to 10 minutes after removal from the incubator. The captured images were analysed using FLIR Systems ThermaCAM Researcher Professional 2.8 SR-1 software.

2.3.2.2 Multiple larvae

A second experiment was carried out using the same method outlined in Section 2.3.2.1, but rather than using individually counted larvae, instead larger estimated quantities of larvae were used. These were placed in 23cl polystyrene cups (Figure 2.3.2.2) and the depth of larvae was measured using a ruler.

Thermal images were taken, as described in Section 2.3.2.1, but up to 100 minutes after removal from the incubator.

The numbers of larvae were counted after the experiment.

- 0.625 cm depth = 166-169 larvae
- 1.25 cm depth = 282-288 larvae
- 2.25 cm depth = 423 larvae
- 4.75 cm depth = 979 larvae

Figure 2.3.2.1 1, 5, 10 and 40 *Calliphora vomitoria* post-feeding larvae in individual cups

Figure 2.3.2.2 Multiple *Calliphora vomitoria* post-feeding larvae in individual cups

2.3.3 Results

2.3.3.1 Individual larvae

Thermal images taken of the larvae less than one minute after being removed from the incubator show that all larvae retained some heat (Figure 2.3.3.1), but 10 minutes after removal from the incubator only the group of 40 larvae still retained some heat (Figure 2.3.3.2).

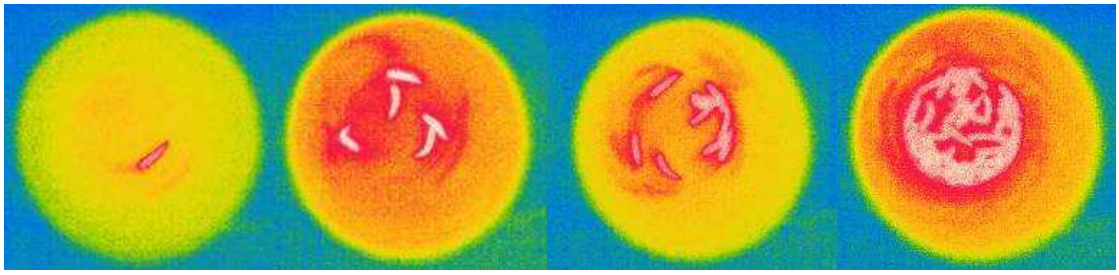


Figure 2.3.3.1 From left to right: 1, 5, 10 and 40 post-feeding *Calliphora vomitoria* larvae filmed by thermal imaging less than one minute after removal from 25°C incubator into room with an ambient temperature of 19°C-21°C

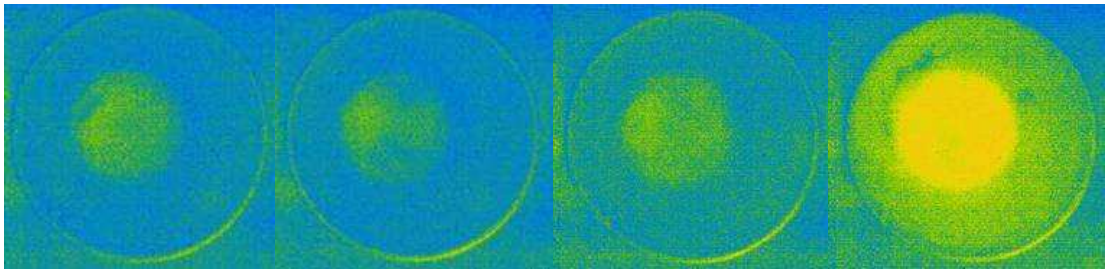


Figure 2.3.3.2 From left to right: 1, 5, 10 and 40 post-feeding *Calliphora vomitoria* larvae filmed by thermal imaging 10 minutes after removal from 25°C incubator into room with an ambient temperature of 19°C-21°C

The results are plotted in Figures 2.3.3.3 (Replicate 1), 2.3.3.4 (Replicate 2) and 2.3.3.5 (combined). When they were first taken out of the incubator, all the larvae were within 1°C of the incubator temperature (25°C). After 10 minutes the temperature of the 1, 5, and 10 larvae had equilibrated to the ambient temperature (21°C), whereas that of the 40 larvae was still 2°C higher than the ambient room temperature.

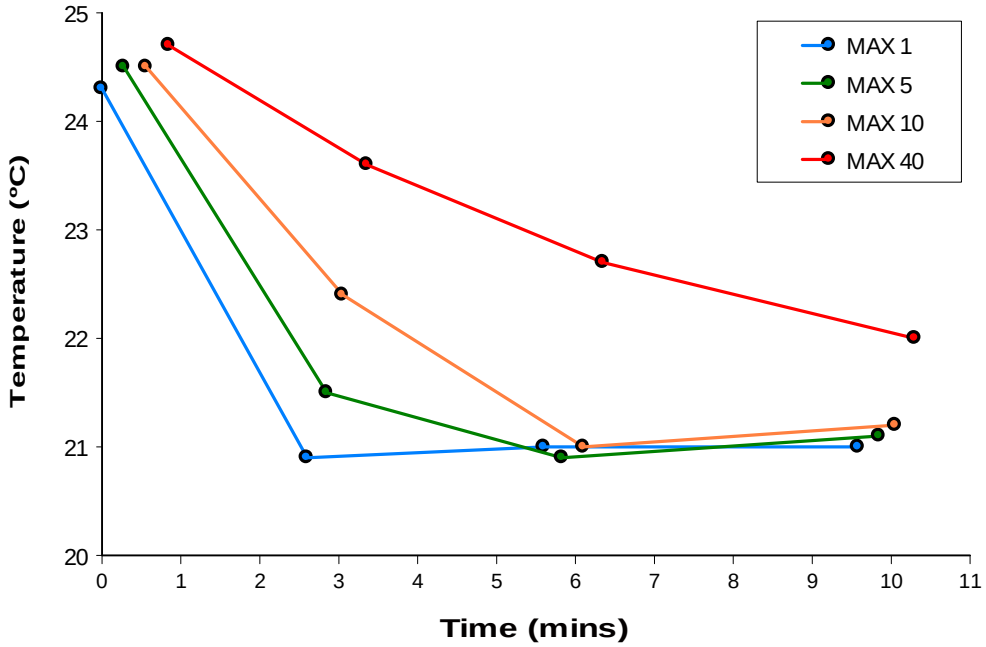


Figure 2.3.3.3 Change in maximum temperature of post-feeding *Calliphora vomitoria* larvae (1, 5, 10 and 40 larvae) after transfer from 25°C incubator to 19°C-21°C room (Replicate 1) [Graph produced by Martin Hall]

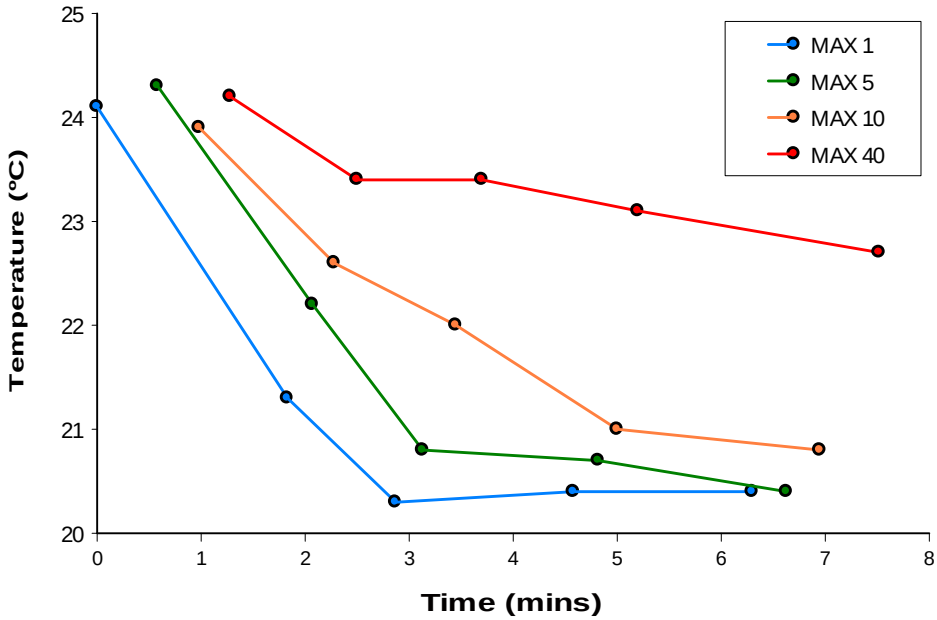


Figure 2.3.3.4 Change in maximum temperature of post-feeding *Calliphora vomitoria* larvae (1, 5, 10 and 40 larvae) after transfer from 25°C incubator to 19°C-21°C room (Replicate 2) [Graph produced by Martin Hall]

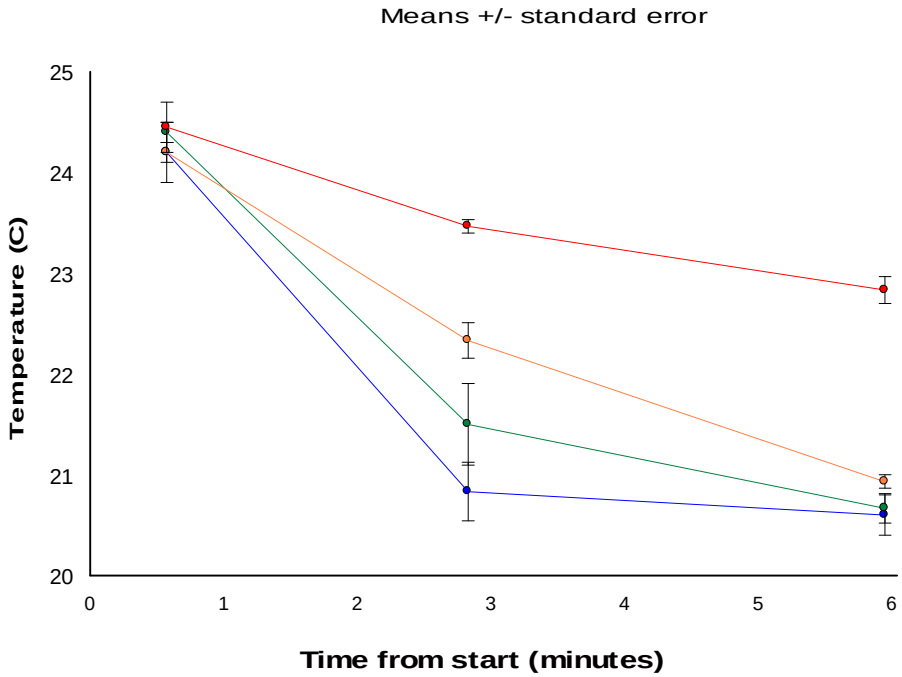


Figure 2.3.3.5 Change in maximum temperature of post-feeding *Calliphora vomitoria* larvae after transfer from 25°C incubator to 19°C-21°C room (Replicates 1 and 2 combined). No. of larvae: 1 (blue), 5 (green), 10 (yellow) and 40 (red) [Graph produced by Martin Hall]

2.3.3.2 Multiple larvae

The changes in temperature over 90-100 minutes are shown in Figures 2.3.3.6 (0.625cm and 1.2cm) and 2.3.3.7 (2.25cm and 4.75cm). All larvae were above the incubator temperature when they were first removed, the greatest number of larvae with the highest temperature, and the smallest number of larvae with the lowest temperature. For all depths of larvae, the temperature dropped by about 2°C after 40-50 minutes, before increasing again to almost the temperature they were at when they were first removed from the incubator. After 90 minutes the larvae retained temperatures above ambient of almost 4.5°C (0.625cm), almost 6.0°C (1.35cm), 5.5°C (2.25cm) and 7.0°C (4.75cm).

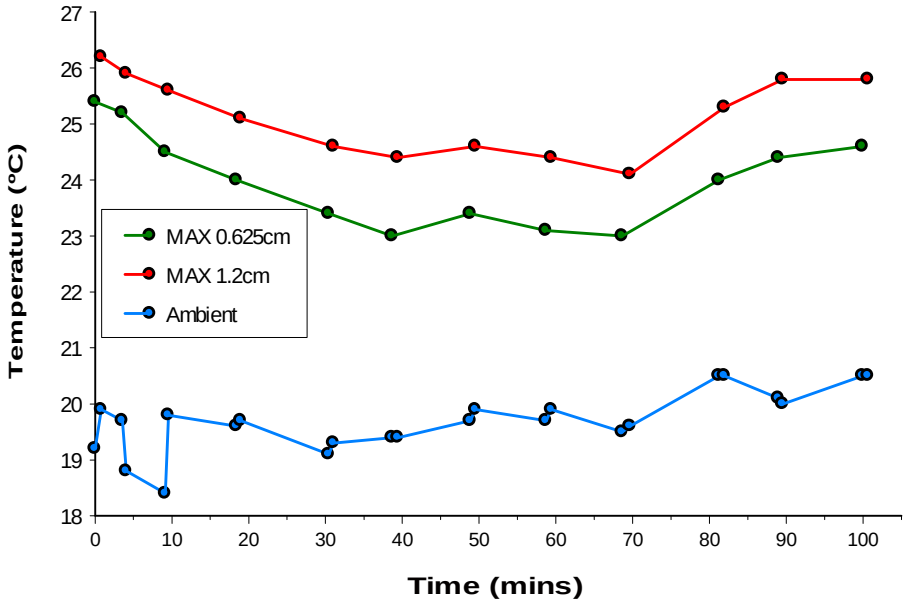


Figure 2.3.3.6 Change in maximum temperature of post-feeding *Calliphora vomitoria* larvae after transfer from 25°C incubator to 19°C-21°C room (0.625cm-1.2cm) [Graph produced by Martin Hall]

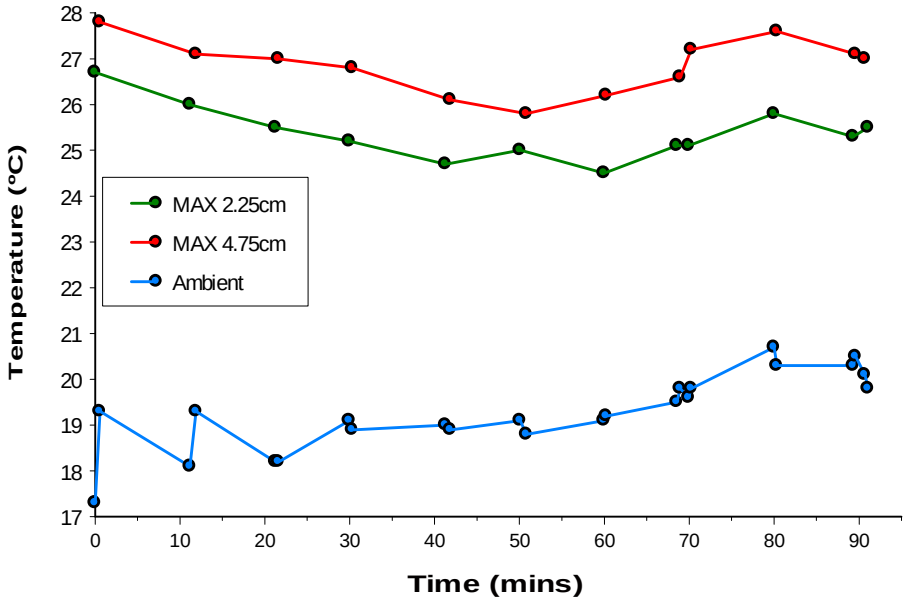


Figure 2.3.3.7 Change in maximum temperature of post-feeding *Calliphora vomitoria* larvae after transfer from 25°C incubator to 19°C-21°C room (2.25cm-4.75cm) [Graph produced by Martin Hall]

2.4 Thermal imaging of piglet cadaver

2.4.1 Aims

The aim was to continue the thermal imaging study described in Section 2.3 carried out on post-feeding larvae, but *in vivo*, i.e. using a piglet cadaver on which there were larval masses feeding and developing. This would allow thermal images to be compared with the decomposition stage, age of the larvae, size of larval masses and internal temperature probes. The hypothesis was that the thermal imaging temperatures would be a good indication of the presence of larval masses inside the cadaver and that the recorded temperatures would reflect those recorded by datalogger probes. There was no replication of this experiment as it was for exploratory purposes as a precursor to later thermal imaging studies.

2.4.2 Materials and methods

A still-born piglet weighing 1000g had previously been purchased from an Oxfordshire pig farmer and stored in a freezer. 24 hours before the start of the experiment, it was wrapped securely in two plastic bags to ensure that no flies were able to oviposit on it prior to the start of the study, and defrosted at room temperature. Once defrosted, the piglet was placed into a plastic tray to contain decomposition fluids and put into a room with the windows left open. Three Tinytag® dataloggers were set to record hourly temperatures: one was placed in the room to record ambient temperature, while the other two had probes attached which were inserted into the mouth and rectum of the piglet cadaver. The Tinytag® dataloggers measure temperatures of -40°C to +85°C, have a reading resolution accuracy of 0.01°C and are recalibrated annually to ensure accuracy (full specification is available at www.geminidataloggers.com).

Despite the windows in the room being open, however, after four days no flies had been seen inside the room and there were no egg masses on the body, therefore eight captive-bred female *Calliphora vicina* (Diptera: Calliphoridae) were released into the room. This was thereafter taken to be the start of the experiment, i.e. Day 1.

The piglet was photographed at 16:00 hours each day for 14 days using a FLIR Systems AGEMA 570 infrared camera and the captured thermal images were analysed using FLIR Systems ThermaCAM Researcher Professional 2.8 SR-1 software. The dataloggers were downloaded using Tinytag Explorer 4.3 and analysed using Unistat 6.0. At the end of the experiment, 100 emerged flies collected from the piglet were identified as *Calliphora vicina*.

2.4.3 Results

2.4.3.1 Larval development

Photographs of the piglet taken on Days 5, 8, 10 and 14 are shown Figure 2.4.3.1. Eggs were laid by the blowflies on Days 1 and 2, and hatched into 1st instar larvae on Days 2 and 3. By Days 4 and 5 they had reached the late 2nd instar stage. From Day 6 onwards they were in the 3rd instar stage and spread throughout the body, moving from the anterior to the posterior end. By Day 12 some of the larvae were in the post-feeding stage and starting to leave the body to pupate. By Day 14, there were no larvae feeding on the body and very little soft tissue remaining, just dry skin and bones. The end weight of the piglet was 120g, therefore there was an 88% loss of tissue body mass.

Figure 2.4.3.1 Photographs of decomposing piglet on Days 5, 8, 10 and 14

2.4.3.2 Thermal images

Thermal images taken of the piglet on Days 1, 5, 7, 8, 9, 10, 12 and 14 are shown in Figure 2.4.3.2. Colder areas are purple, the hotter areas are yellow.

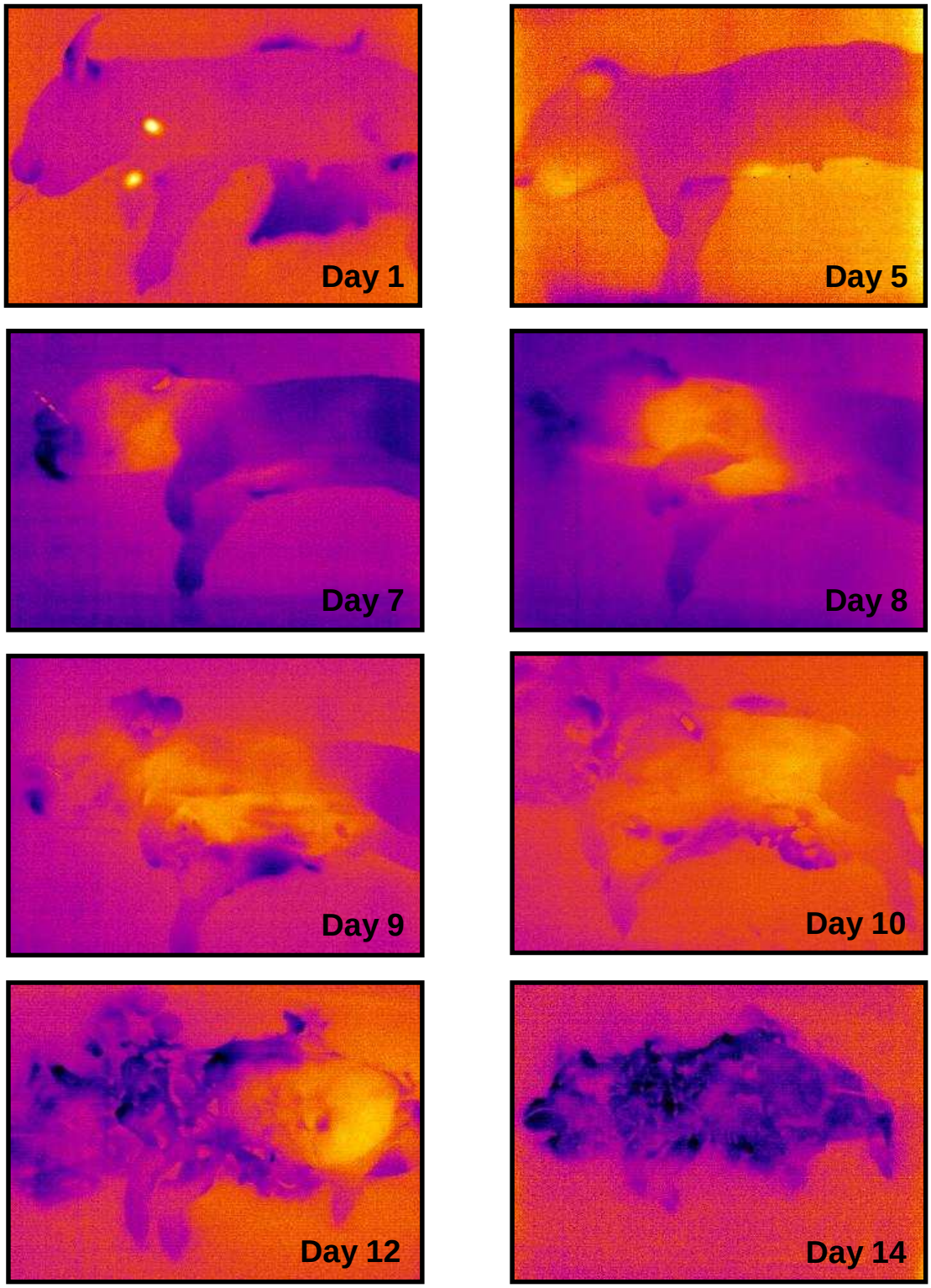


Figure 2.4.3.2 Larval masses in piglet visible by thermal imaging. Images taken on Days, 1, 5, 7, 8, 9, 10, 12 and 14. Colour spectrum: purple=cold yellow=hot

On Day 1, two “hot” adult flies can be seen on the body. No increased temperatures were visible in the thermal images until Day 5, when warmer areas could be seen in the chin and chest region of the piglet. By Day 7, a larval mass was clearly visible in the neck of the piglet, which then moved down the body over the following days. By Days 9 and 10, the larval mass had increased in size and spread down through the abdomen. By Day 12, the larval mass was located in the posterior end of the body, while the anterior end had become skeletonised. By Day 14 there was no larval mass visible in the body.

Each thermal image was viewed using FLIR software, illustrated in the screenshot shown in Figure 2.4.3.3. The image of the piglet, taken on Day 8, is shown together with various temperature data: minimum (10.3°C) and maximum (16.7°C) temperatures for the whole image, and the difference between them (6.4°C); two spots, SP01 (12.0°C) and SP02 (16.3°C); a boxed area AR01 (minimum 14.2°C, maximum 16.7°C, difference 2.5°C); and a line LI01 (minimum 11.4°C, maximum 16.5°C, difference 5.1°C) and a graph of the line below.

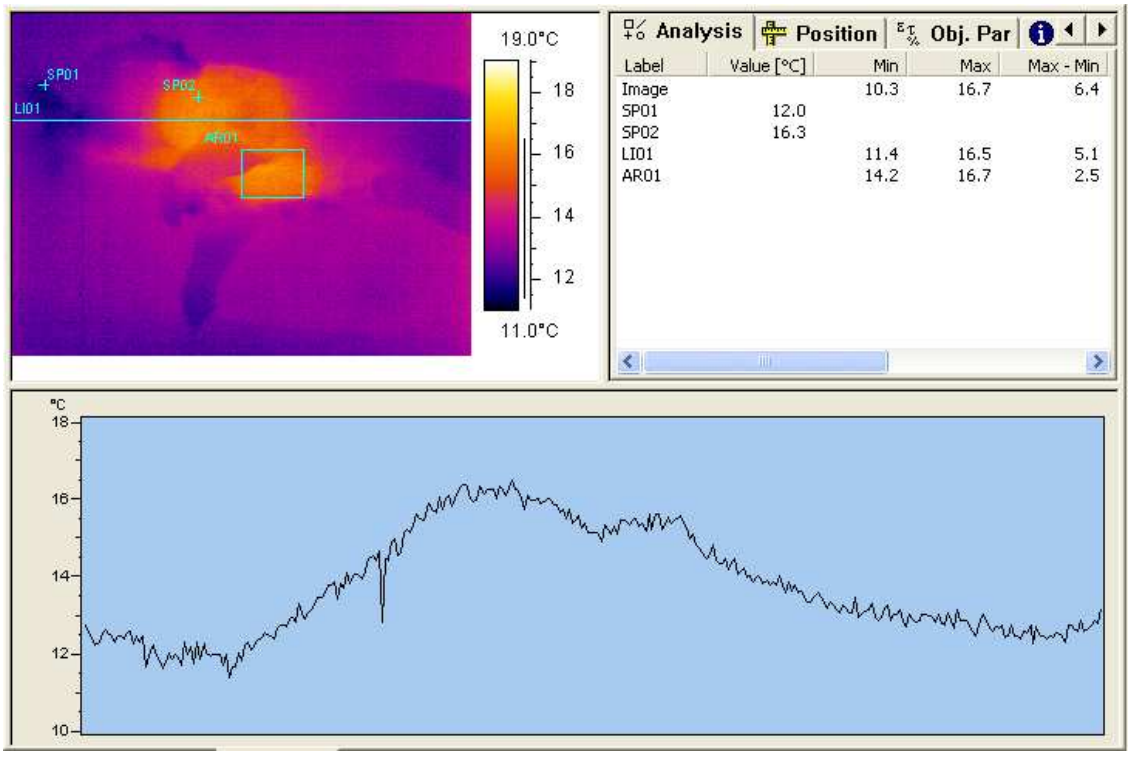


Figure 2.4.3.3 Analysis of thermal image taken on Day 8, indicating the temperatures in two spots (SP01 & SP02), within a rectangular area (AR01) and along a line across the piglet (LI01), the latter being shown graphically below the image with temperature in °C on the vertical axis.

For each day of the experiment, from Days 1 to 14, a single thermal image was chosen which showed the entire body length of the piglet, and a line drawn from anterior to posterior, as illustrated by LI01 (Figure 2.4.3.3), and the data for each line are summarised in Table 2.4.3.1. Temperatures along the line from the anterior to posterior of the piglet ranged from 10.1°C to 17.1°C throughout the 14 days of the experiment. The maximum temperature difference across the cadaver was 5.1°C on Day 8.

Table 2.4.3.1 Maximum, minimum, mean and range of temperatures recorded from line drawn across thermal images taken of a piglet cadaver daily, from Day 1 to Day 14

Day	Minimum temp (°C)	Maximum temp (°C)	Mean temp (°C)	Range of temp (°C)
1	15.0	16.2	15.6	1.2
2	15.1	16.4	15.6	1.3
3	14.0	15.1	14.6	1.2
4	12.4	13.9	13.1	1.6
5	13.8	15.4	14.5	1.6
6	14.5	17.1	15.3	2.6
7	11.3	14.9	12.7	3.6
8	11.5	16.6	14.0	5.1
9	10.2	14.1	12.8	3.9
10	11.4	15.3	13.2	4.0
11	10.1	14.0	12.0	3.8
12	12.1	15.9	13.4	3.8
13	13.0	15.1	13.9	2.0
14	12.6	14.4	13.8	1.8

2.4.3.3 Temperatures

The hourly temperatures recorded by the three dataloggers are plotted in Figure 2.4.3.4, together with the maximum temperatures recorded by thermal imaging at 24 hour intervals. The ambient temperature fluctuated diurnally for the first three days, averaging around 15°C. From Day 3 to Day 6 the room temperature stabilised at around 14°C, but then dropped steadily to 11.2°C by Day 9. From there it rose again, reaching 15.1°C on Day 14. The temperatures recorded by the probe dataloggers inserted in the mouth and rectum of the piglet followed a similar pattern to the ambient temperatures, although the temperatures in the mouth were higher than those in the rectum until Day 9. The highest temperature in the mouth was 18.9°C on Day 6. Thereafter it fell gradually, although still reflecting the diurnal pattern of the ambient temperature. The rectal temperature peaked on Days 10 and 12 at 16.5°C.

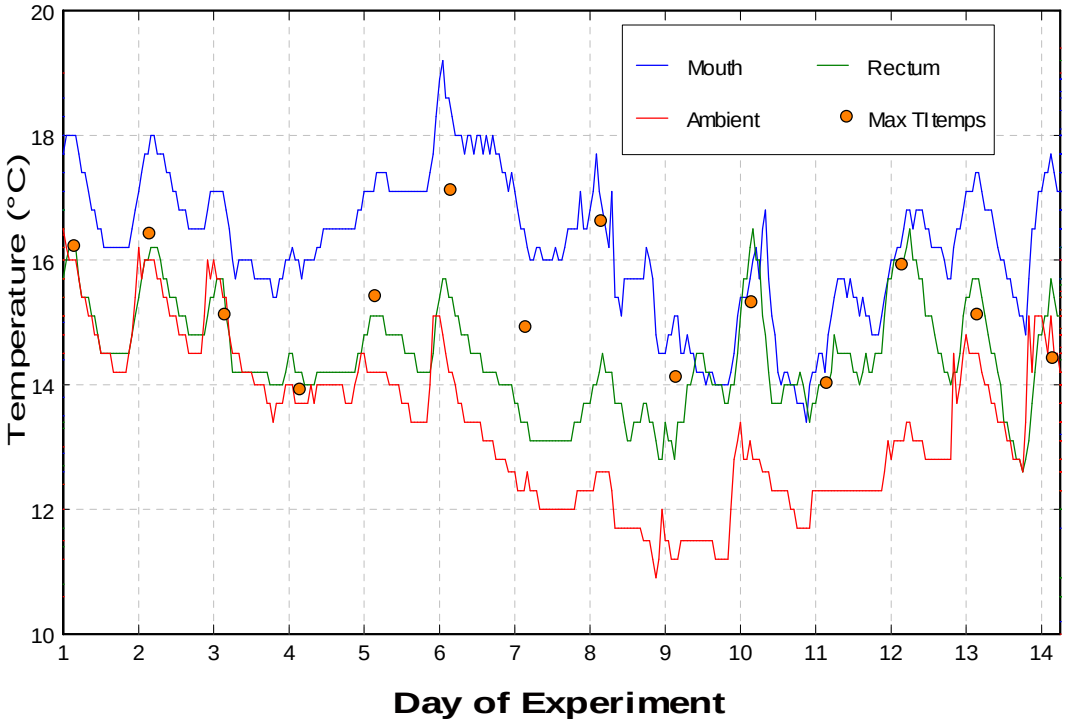


Figure 2.4.3.4 Temperatures recorded at 16:00 each day from Day 1 to 14 of ambient room temperature (red), piglet mouth (blue) and rectal (green) temperatures using dataloggers, and maximum thermal imaging temperatures (orange circles). Day of Experiment at marked at 12:00 midday

The internal datalogger temperatures are plotted again in Figure 2.4.3.5, but showing temperatures above the ambient, together with the thermal imaging

temperatures. Initially there was a 2°C difference between the mouth and rectal temperature, the latter being lower and less than 0.5°C above ambient. After Day 4, the gap increased between mouth and rectal temperatures, and by Day 7 there was almost 4°C between the two dataloggers, the temperatures in the mouth being higher. They remained as high as 5°C above ambient until Day 9. From then until Day 11, the mouth and rectal temperatures were almost the same. From Day 12 onwards, the rectal temperature dropped significantly, while the mouth temperature remained above ambient by a mean of around 3°C. The thermal imaging temperatures initially started closer to the rectal temperatures, until Day 6 when they increased to a similar temperature to the mouth, before dropping to midway between mouth and rectal temperatures on Day 7, before they rose again to 4°C above ambient on Day 8, the same as the mouth temperatures. Thereafter they dropped again, reaching equilibrium with the ambient temperature on Day 14.

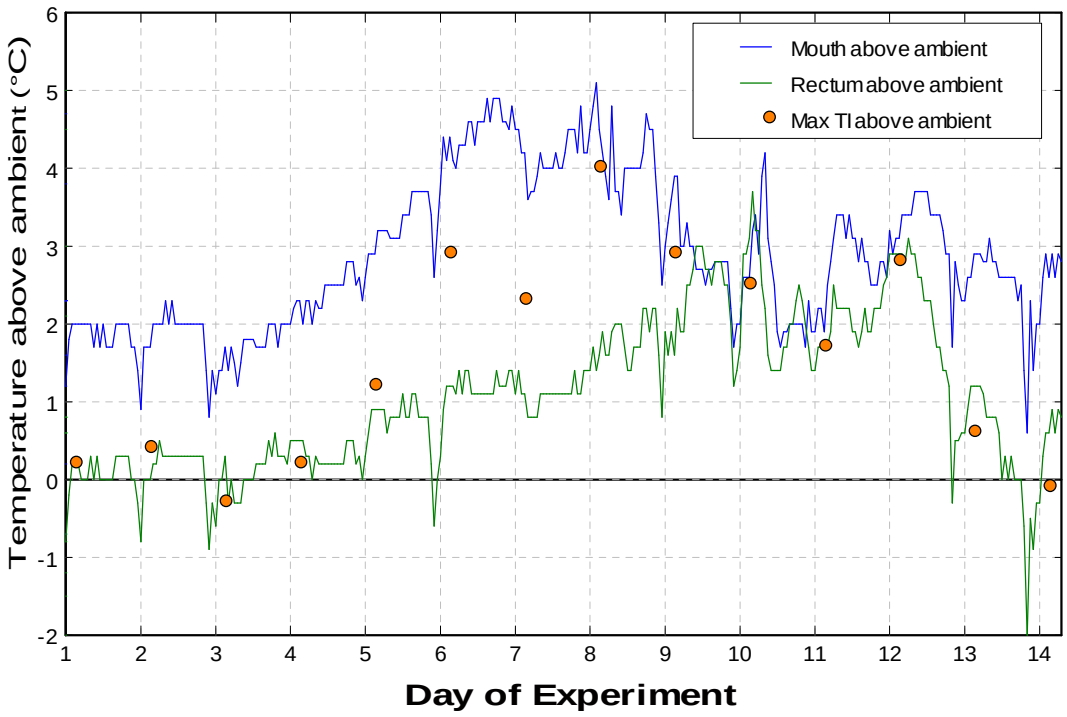


Figure 2.4.3.5 Temperatures above ambient recorded at 16:00 each day from Day 1 to 14 piglet mouth (blue) and rectal (green) temperatures using dataloggers, and maximum thermal imaging temperatures (orange circles). Day of Experiment at marked at 12:00 midday

2.5 Discussion

In the larval mass experiments, just 20 larvae had the effect of raising the local temperature by 1°C above ambient in both Experiments 1 and 2. The highest temperature reached above ambient was up to 5°C for 400 larvae in Experiment 1 and up to 4.5°C for 1000 larvae in Experiment 2. In Experiment 1, 200 larvae reached almost 4°C and in Experiment 2, batches of both 100 larvae and 500 larvae reached 2°C above ambient. The average ambient room temperature in Experiment 1 was 7°C cooler than in Experiment 2, which may account for the slightly higher larval masses temperatures despite the smaller numbers of larvae, as in warmer ambient temperatures the larval mass temperatures may be lower. Studies on two species of *Chrysomya* showed that some elevation of temperature occurs even at low population densities (Goodbrod & Goff, 1990), a finding supported by the results found here. Marchenko (2001) also suggested that the number of larvae influences the magnitude of increased temperature of a larval mass, relative to ambient. This was also supported by the thermal imaging studies carried out, where a mass of 40 larvae retained heat of 1°C above ambient after 10 minutes out of the incubator, whereas 1, 5 and 10 larvae did not retain any additional heat. In the thermal imaging experiments using multiple larvae, all larvae retained 4.5-7°C above ambient temperature after 90 minutes out of the incubator, despite being post-feeding larvae rather than being still in the feeding stage.

The numbers of larvae per gram of food was varied in these experiments, in order to test for the effect of crowding and reduced food source on larval development. Ireland & Turner (2006) noted that increased competition, i.e. larval crowding, resulted in increased development rates and undersized larvae and adults. Likewise in these experiments larvae exposed to severe competition, i.e. 1g food : 100 larvae, had lower pupal sizes and weights than those larvae exposed to an adequate food resource. In addition, the adult wing vein dm-cu was marginally shorter in this group. The culture with only 20 larvae took far longer to complete the feeding stage and enter the pupariation stage, most likely due to the lower larval mass temperatures, compared to the other

cultures. Experiments carried out by Saunders and Bee (1995) suggested that 1g food per single larvae allows sufficient development, but 50-150 larvae per 50g of food resulted in reduced pupal weight and adult size.

A critical food shortage has previously been demonstrated to increase the duration of the larval stage for some species (Ullyet, 1950; Kamal, 1958), however, that finding was not supported here, where the reduced food resource resulted in smaller pupae and adults, but did not increase the duration of the larval stage. The survival rate of all cultures was affected, with mortality being higher in Experiment 2 (20%-52% to pupal stage, 48%-90% to adult stage) than in Experiment 1 (8%-24% to pupal stage, 28%-36% to adult stage). The reason for this is unclear, but may have been due to the higher ambient temperature, +7°C, in Experiment 2, resulting in lower humidity and therefore desiccation of the pupae.

Developmental studies carried out on batches of 20 *Calliphora vicina* larvae at a range of temperatures estimated that the maximum larval length would be reached after around 3000 ADH (Donovan *et al.*, 2006). In Experiment 2, larvae reached their full length, or post-feeding stage, after 2800 ADH, which is consistent with their findings.

Thermal imaging on the piglet also clearly showed that the larvae laid as eggs in the mouth moved down through the body as they continued feeding and growing. Therefore they were feeding on many different body tissues, including brain, liver and muscle. Laboratory studies have suggested that different food types result in differences in developmental rate (Ireland & Turner, 2006), but on smaller carcasses such as piglets or young children, these data should be treated with caution, as the larvae will be consuming a variety of soft tissues available to them. The use of a thermal camera, however, could be used to indicate the areas of the body in which the larvae are feeding, e.g. head, abdomen, genitals etc.

The thermal imaging studies carried out on a piglet also showed a maximum thermal mass temperature above ambient of 5°C, recorded by the probe inserted into the mouth of the piglet. The mouth and rectal probes followed the fluctuating temperatures of the ambient room temperature, although the mouth probe remained 2°C higher than the ambient for the first three days. Thereafter it increased at a fairly steady rate. Although the mouth probe reached the highest temperature on Day 6, ~19°C, the highest temperature above ambient was actually reached on Day 8. This was also reflected in the thermal imaging measurements, although the maximum above ambient recorded was 4°C. The rectal temperatures remained close to ambient until about Day 5, when they also started to rise, although the maximum above ambient was <4°C on Day 10.

The thermal imaging technique was advantageous in two main ways. Firstly, it enabled the temperatures of individual larvae to be recorded by video, ensuring that continuous temperature recording could be made without the use of invasive probes. Secondly, it enabled the location of larval masses to be seen within a cadaver, even when they were not visible from the outside. The maximum temperature of the larval masses measured with thermal imaging was approximately 1°C lower than those recorded using the internal datalogger probes, perhaps because the thermal imaging camera was measuring the surface temperature of the pig rather than the larval masses within it. In the studies using post-feeding larval masses, at different depths, the temperatures were recorded using only the thermal imaging camera, and without using a probe. Further studies could therefore be undertaken measuring larval masses at different depths using both methods, to ascertain to what depths the larval imaging camera can accurately record temperatures.

The results of these experiments supported the hypotheses: that even small numbers of feeding larvae are able to generate a larval mass temperature higher than the ambient temperature; that increased crowding will result in smaller individuals, through to the adult stage; that thermal imaging can be used successfully to track and record larval mass temperatures both in laboratory cultures and cadavers.

CHAPTER 3

PIGLET DECOMPOSITION

3.1 Introduction

Temperature is the main determining factor in the developmental rate of insects, and specifically, the developmental rate of Calliphorid larvae (Slone & Gruner, 2007), and this is crucial in determining the post-mortem interval of cadavers in forensic entomology casework. In warmer temperatures the larvae will develop faster, and in cooler weather they will develop slower. This is what would be expected in an outdoor, natural environment. Indoors, however, a cadaver may be buffered from the extremes of temperature, therefore the extreme higher and lower temperatures would not be experienced by an indoor cadaver (unless air conditioning or heating were a significant contributor). Another limiting factor is the accessibility of the adult fly to the cadaver, which may result in a delay of colonisation on a body. In a study comparing three indoor and three outdoor pig carcasses, Anderson (2011) reported a five day delay in indoor carcasses being colonised even though the mean temperature was 16.5°C (range 4-43°C) outdoors and 17.8°C (range 11.5-40°C) indoors, resulting in greater larval numbers and hence faster decomposition on the outdoor carcasses. In a series of nine pairs of piglets, of which one was placed indoors and one outdoors, Reibe & Madea (2010) reported a 24 hour delay in oviposition on seven out of nine indoor pigs. The temperature was about 2°C warmer indoors, but the smaller egg masses resulted in less larval load and slower decomposition. In addition, some species of blowfly may favour either indoor or outdoor remains. Anderson (2011) observed the same five blowfly species colonising both indoor and outdoor carcasses, with an additional two species attracted to the outdoor carcasses. In contrast, Goff (1991) reported that out of 35 forensic cases investigated on the island of Oahu, Hawaii, only five insect species were found in both indoor (n=14) and outdoor (n=21) scenes out of a total of 22 recorded insect species: the majority of Diptera species were indoor, and the majority of Coleoptera species were outdoor.

Few published studies on the succession of cadaverous insects have been carried out in a natural environment in the UK, and certainly not to the extent of those carried out in the USA using pigs (*Sus scrofa domestica*) on land (Payne, 1965), in water (Payne & King, 1972) and buried (Payne *et al*., 1968a). Lane (1975) carried out succession studies using four vole (*Clethrionomys glareolus* Schreber, 1780) carcasses at Silwood Park, Berkshire, although these were exposed for a period of only 19 hours and were then kept in fly-proof cages for a further 50 hours, therefore recording initial oviposition by blowflies rather than the succession of arthropods over a longer period. Smith & Wall (1997) also studied Dipteran succession on small carcasses, in the South West of England, using a total of 180 mice (Muridae spp.) and 143 quail (*Coturnix coturnix* Linnaeus, 1758) in three separate trials, removing one third of the carcasses to the laboratory after two, five and ten days. Hwang & Turner (2005) also studied dipterous assemblages from six different sites in the UK over a 30km transect, ranging from urban Central London to rural Surrey to the South-West, but using pigs' liver rather than complete corpses. Easton (1966) studied the decomposition of a dead fox (*Vulpes vulpes* Linnaeus, 1758) in the UK, although this study was concentrated on the Coleoptera species colonising the body. The only study so far in the UK to have identified the complete succession of arthropods on a complete cadaver, recording Diptera, Coleoptera, Hymenoptera, Collembola and Arachnida was carried out by Smith (1975) who studied the complete decomposition of a dead fox for more than three months through to the skeletal stage. An unpublished study was also carried out by LeBlanc (2008) following insect succession on three pigs: one for one month (October to November), one for three months (July to October) and one for five months (May to October).

The life cycle of blowflies is completed by their emergence as adults from the puparium, which is formed by the constriction, hardening and pigmentation of the outer skin of the post-feeding 3rd instar larvae. It is within this hard pupal case that the pupa then forms and metamorphoses into the adult fly. In some forensic cases, the only insect evidence remaining at the site where a body has decomposed may be the empty pupal cases, if blowflies have completed their development and already emerged as adults. These puparia can still be of

forensic use because the most common species of blowfly can be identified from the larval mouthparts, posterior spiracles and abdominal spines which are still visible (Smith, 1986). They can therefore be used to give a minimum time since death, i.e. one complete generation, or to indicate the season in which the body was colonised (Gilbert & Bass, 1967).

There is, however, scant information available regarding the length of time that puparia will survive in the environment before they disintegrate, and reports are generally related to archaeological sites (Gilbert & Bass, 1967; Teskey & Turnbull, 1979) or exhumations (Ardő, 1953). In addition, these often refer to non-Calliphorid Diptera such as the so-called Coffin Fly, *Conicera tibialis* Schmitz (Phoridae) (Ardő, 1953). The empty puparia of a blowfly, *Phormia terraenovae*, were, however, found in the close vicinity of a skeleton of a person who, it later transpired, had died four years earlier, suggesting that puparia may survive for up to four years “in normal outdoor conditions” (Nuorteva, 1987).

Two years after the skeletal remains of a body was recovered in Southern England, forensic entomologists at the Natural History Museum were asked to determine if the body had decomposed in the same place, i.e. whether they could find any remaining insect evidence to indicate this. After an intensive search of the area, fewer than half a dozen blowfly puparia were recovered in the vicinity, therefore no conclusion could be drawn, although this raised the possibility that the wrong location had been searched, or that active decomposition had not occurred there and therefore there would be no remaining empty puparia. If it was, however, the correct location and if active decomposition of the body had occurred at the site, one possibility is that blowfly puparia had been present two years before but had since disintegrated. Without knowing how long blowfly pupal cases are likely to survive in a natural UK outdoor environment, it was not possible to answer the question of whether active decomposition of the body had taken place there.

3.2 Indoor vs. outdoor insect succession and blowfly larval development

3.2.1 Aims

The aim of this study was to compare the decomposition of pairs of indoor and outdoor piglets at different times of the year. Factors which were considered were accessibility of the cadavers to blowflies, i.e. rate of oviposition, species of blowflies attracted to the cadavers, larval development rate, larval mass elevated temperatures, ambient temperatures and rate of decomposition. The hypotheses being tested were that oviposition would be delayed on the indoor pigs, but that ambient temperature would have a more significant affect on the outdoor cadaver. The minPMIs were also estimated and compared with published data.

3.2.2 Materials and methods

3.2.2.1 Study site

The Natural History Museum in South Kensington, London was the site used for this study. The indoor location was a disused office with partially open windows on the 6th floor of the building. Approximately 20m away was a rooftop the same height from the ground which was used as the outdoor location.

3.2.2.2 Animal model

Ten stillborn piglets were acquired from a pig farmer in Oxfordshire and laid out in pairs, one indoor and one outdoor, in five separate “runs”, as per the protocol used by Reibe & Madea (2010), between December 2003 and October 2005. As the piglets were obtained stillborn, their weights/sizes could not be controlled, but each pair of piglets was paired in similar weights where possible. The experimental areas were separated by a short staircase, two internal doors and one external door, the carcasses being approximately 40m apart. The

indoor piglets were placed on a shelf in a plastic bowl to contain decomposition fluids (Figure 3.2.2.1), and prior to the post-feeding larval stage, the bowl was placed in a large box filled with sand into which larvae could disperse and pupariate. The outdoor piglets were placed in a plastic bowl and then enclosed inside a collapsible metal dog cage to guard against vertebrate scavengers such as rats and crows. The cages measured 61cm height x 61cm width x 122cm length, with a hinged door at one end. The cages were secured to the ground with rope and plastic was placed over the top as protection against the rain (Figure 3.2.2.2). The distance between the indoor and outdoor pigs was approximately 20m, separated by two doors.

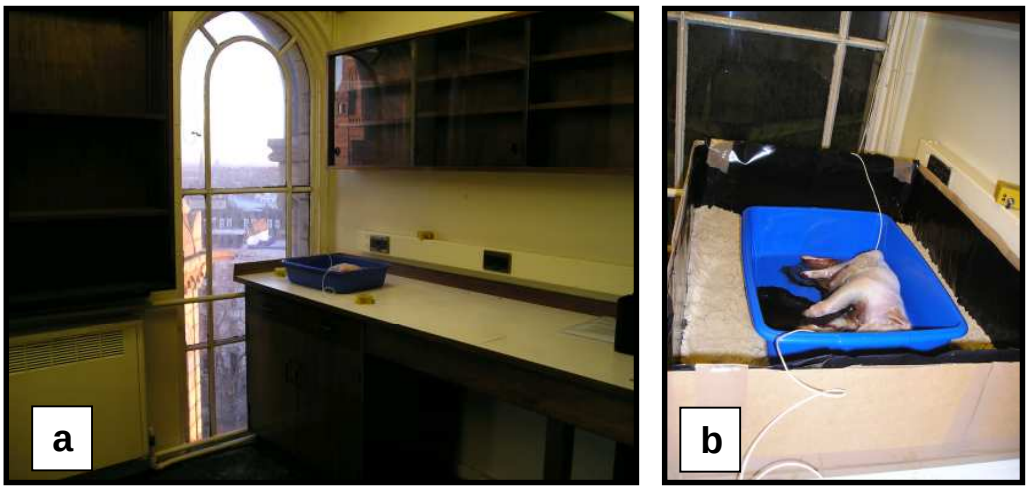


Figure 3.2.2.1 Set-up for indoor piglet cadaver in disused office in NHM (**a**) in plastic bowl for larval feeding stage, and (**b**) plastic bowl placed in sandbox for post-feeding and pupal stages

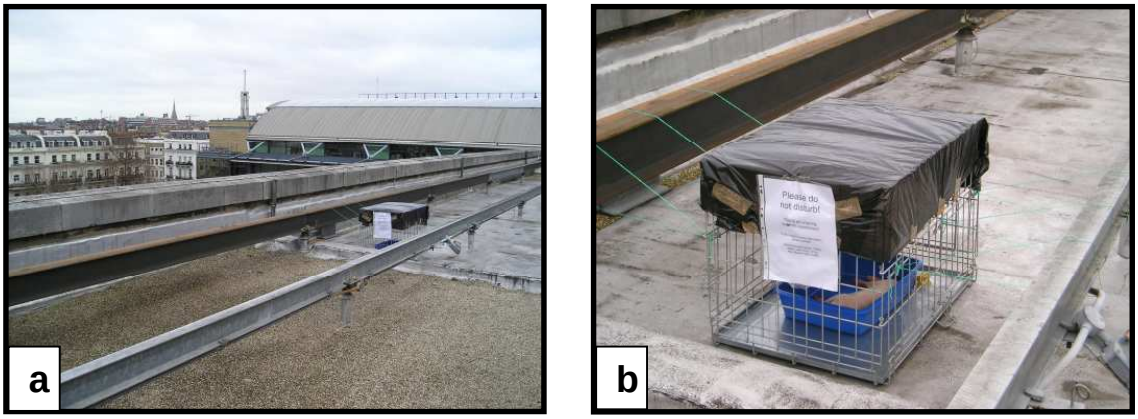


Figure 3.2.2.2 Set-up for outdoor piglet cadaver (**a**) on rooftop at NHM, and (**b**) in plastic bowl placed in metal dog cage and covered with black plastic sheet

3.2.2.3 Sampling procedure

Tinytag® Plus datalogger probes were inserted into the mouth and rectum of each piglet to record hourly internal temperatures. A Tinytag datalogger was also placed close to each piglet to record hourly ambient temperatures during the duration of each run. All piglets were weighed at the start and end of each run to measure percentage weight loss. Each run was started on Day 1, and thereafter each piglet was visited daily while fly larvae were developing on the body, and thereafter at intervals of approximately once every three days. All the piglets are listed in Table 3.2.2.1, together with their starting weights, and the start and end date of each run.

Table 3.2.2.1 List of 10 piglets used for indoor (i) and outdoor (o) experiments, their starting weights (g), and dates of experiments

Run No.	Piglet	Start weight (g)	Start date	End date
1	1i 1o	1500 2000	12th Dec 2003	25th Feb 2004
2	2i 2o	2640 1290	27th Feb 2004	16th April 2004
3	3i 3o	560 300	22nd May 2004	18th June 2004
4	4i 4o	880 800	2nd March 2005	14th April 2005
5	5i 6o	1380 1320	2nd Aug 2005	19th Oct 2005

At each visit, the piglets were photographed and notes were made regarding stage of decomposition, insect activity and weather conditions, including wind, rain and cloud cover (Table 3.2.2.2). Larval mass temperatures were recorded using a TN1 non-invasive infrared thermometer, which has a temperature range of -33°C to 220°C, a resolution of 0.1°C and accuracy of $\pm$2% or $\pm$ 2°C, whichever is greater (full specification at http//thermometer.co.uk).

At each visit, samples of the larvae were collected, killed in freshly boiled water and placed in 80% ethanol in runs 3, 4 and 5. Ten larvae from each sample were identified and their life stage determined under a binocular microscope up to x50 magnification using a standard identification key (Smith, 1986) and their length was measured using an eyepiece graticule.

Table 3.2.2.2 Codes used whilst taking notes of weather conditions for indoor and outdoor piglet experiments

Element	Code	Description
Wind	W0	No wind
	W1	Slight breeze
	W2	Gusting
	W3	Gales
Rain	R0	No rain
	R1	Drizzle or small showers
	R2	Regular showers
	R3	Heavy and constant rain
Cloud	C0	No cloud, blue skies, sunshine
	C1	Light cloud, some sunshine
	C2	Mainly cloudy, a bit of sunshine
	C3	Very overcast, no sun all day

3.2.3 Results

The biomass loss of each piglet is shown in Table 3.2.3.1 and Figure 3.2.3.1, except for Piglets 2o and 3o where a significant proportion of the carcasses were taken by birds and therefore the remaining mass could not be accurately calculated. Seven of the remaining eight piglets showed a biomass loss of more than 80%, which could be attributed almost entirely to blowfly activity.

Table 3.2.3.1 List of 10 piglets used in indoor (i) and outdoor (o) experiments showing loss of biomass

Run No.	Piglet	Start wt (g)	End wt (g)	Wt loss (g)	Wt loss (%)
1	1i	1500	150	1350	90.0
	1o	2000	140	1860	93.0
2	2i	2640	480	2160	81.8
	2o	1290	Not known	Not known	Not known
3	3i	560	150	410	73.2
	3o	300	Not known	Not known	Not known
4	4i	880	80	800	90.9
	4o	800	140	660	82.5
5	5i	1380	150	1230	89.1
	6o	1320	140	1180	89.4

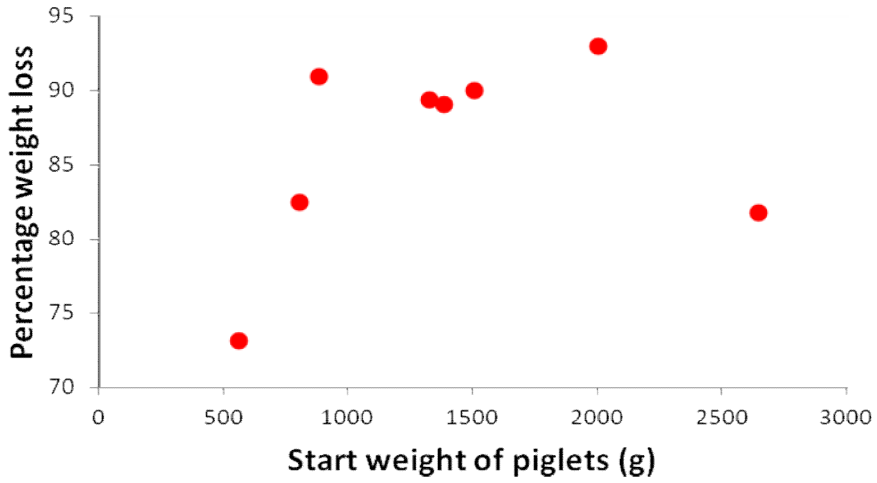


Figure 3.2.3.1 Loss of biomass for eight piglets used in indoor (i) and outdoor (o) experiments showing start weight (g) and percentage weight loss by the end of the experiment

The following sections, 3.2.3.1 to 3.2.3.5, outline the results for each run: field observations, photographs, dataloggers statistics, dataloggers graphs, infrared temperature graphs, decomposition and blowfly stages and larval development graphs (the latter for runs 3, 4 & 5 only). The combined results of all five runs are outlined in section 3.2.3.6.

3.2.3.1 Run 1

Table 3.2.3.2 Run 1: Field observations recorded at each visit to indoor and outdoor piglets

Day	Date & time	Weather	Piglet 1i (indoor)	Piglet 1o (outdoor)
1	12th Dec 2003 16:00 [started dataloggers]	W2/R1/C2	Marbling around face	Marbling around face
2	13th Dec 2003 16:00	W2/R1/C2	No flies, marbling of abdomen	Single Calliphorid on upper body
3	14th Dec 2003 15:00	W2/R0/C1	2 Calliphorids around back legs & anus and buzzing at window; 1 female looking for oviposition site; eggs laid under chin	No flies; egg masses in exposed ear, on elbow and under front leg
4	15th Dec 2003 16:00	W1/R0/C0	No obvious change; no flies; small egg masses on neck and top of front legs	Slight sagging on abdomen
5	16th Dec 2003 16:00	W1/R0/C1	1st instar larvae under body; still eggs around neck; larvae feeding on seeped blood in bowl; seepage of liquid from orifices – ears, nose, anus, mouth, eyes; eggs laid on inside of forelegs and on back leg behind knee in fur; lots of 1st/2nd instar larvae between front legs in blood; 1 adult fly in room	[No comments]
6	17th Dec 2003 16:00	W1/R0/C0	Bloated; 1 calliphorid; egg masses in ear, on neck & hind legs; 2nd instars under neck, stomach and between hind legs	*Calliphora* ovipositing under neck; no eggs hatched
7	18th Dec 2003 16:00	W1/R0/C0	Still bloated, esp. legs; lots of flies laying new egg masses on legs, behind ear, in cheek & face; 1st/2nd instar masses in snout (mean 4.5mm), neck (mean 9.9mm) & between hind legs (mean 10.6mm); foaming from chest & skin falling away from stomach	4 *Calliphora* seen in morning; new egg mass between front legs; no bloating; no larvae visible
8	19th Dec 2003 17:30	W0/R0/C2	Fly laying eggs on cheek; 1st instars on snout & in ear	Fly laying eggs on back and under body
9	20th Dec 2003 16:00	W2/R1/C3	No flies; maggot masses now inside body, barely visible except under skin; maggot mass at rear end	No flies
10	21st Dec 2003 16:00	W2/R0/C1	Back end completely collapsed; maggots are 3rd instar, under skin of belly & chest; 1st instars in ear and around snout; more egg masses on neck	[No comments]
11	22nd Dec 2003 16:00	W2/C3/R0	Lots of decomp fluids; internal organs exposed; larvae visible in rectum	Small sample of eggs taken inside
12	23rd Dec 2003 11:15	W1/R1/C3	Both probes dislodged by larvae, inserted back into centre of larval mass	Eggs taken from body yesterday have hatched

16	27th Dec 2003 16:00	W1/R1/C2	Just small part of torso remaining; lots of gunge; lots of 3rd instar larvae and pupa in various stages; new egg masses in neck & stomach	[No comments]
28	8th Jan 2004 16:00	W2/R1/C2	Older pupae all outside bowl, lighter coloured ones inside bowl; not as much liquid, therefore couldn't crawl out; some 2nd instars in legs; single fly seen in room, entered by window, not newly emerged	Lots of liquid in bowl, more water/rain than blood; some 2nd/3rd instar maggots in bowl, possibly hatched from back/bowl interface; none seen in mouth or ears
29	9th Jan 2004	[Not noted]	Total of 2902 puparia collected	[No comments]
31	11th Jan 2004	W3/R1/C2	No change; some 2nd instar maggots under legs & skin; but no flesh left	Body looking more bloated; odour; new egg mass on neck; internal maggot mass?
32	12th Jan 2004	W3/R1/C3	[No comments]	Body much more sunken in abdomen & head
35	15th Jan 2004	W2/R1/C3	[No comments]	Abdomen very sunken; lots of gunge around rear end; head still intact
39	19th Jan 2004	[Not noted]	First 3 batches of flies have emerged	Snout gone; large Calliphorid laying egg mass on neck; strong odour
40	20th Jan 2004	W0/R1/C3	[No comments]	Lots of new egg masses on neck in folds of skin; rear end collapsed; maggot mass visible
42	22nd Jan 2004	W1/R2/C3	[No comments]	Rear end disintegrated; maggots in wandering stage
44	24th Jan 2004	[Not noted]	Emerged flies all *Calliphora vicina*	3 Calliphorids feeding on body (not ovipositing)
46	26th Jan 2004	W3/C3/R0	[No comments]	Alot of the gunk has dried up; 1 adult Calliphorid seen
55	4th Feb 2004	W3/R1/C2	[No comments]	Took liquid out of bowl; collected wandering maggots and puparia, some up to 15m away in gravel
56	5th Feb 2004	W2/R1/C3	[No comments]	Picked up more puparia from gravel and from under cage
61	10th Feb 2004 16:00	W1/R0/C3	[No comments]	Puparia collected from sand and put into jar
63	12th Feb 2004 16:30	W0/R0/C0	[No comments]	New egg masses laid under legs & inside crevice of abdomen; 2 new emerged *Calliphora vicina*
66	15th Feb 2004 15:00	[Not noted]	[No comments]	Collected more puparia from sand; more newly emerged flies; larvae could be heard feeding inside carcass
76	25th Feb 2004 [removed dataloggers]	[Not noted]	[No comments]	3 adult flies collected from under bowl; more wandering maggots
81	1st March 2004 16:00	W0/R0/C1	[Total of 3988 puparia collected]	3 newly emerged *Calliphora vicina*; puparia and migrating larvae

Figure 3.2.3.2 Photographs taken during decomposition of indoor piglet in Run 1 (Piglet 1i), stating day and date of experiment

Figure 3.2.3.3 Photographs taken during decomposition of outdoor piglet in Run 1 (Piglet 1o), stating day and date of experiment

Table 3.2.3.3 Run 1 datalogger statistics (Day 1 14:00 to Day 20 12:00)

Run 1		Min (°C)	Max (°C)	Range (°C)	Mean (°C)	SD
Indoor	Ambient	11.2	16.0	4.8	14.1	±1.0
	1i mouth	9.0	18.6	9.6	14.5	±1.8
	1i rectum	8.5	17.1	8.6	14.2	±1.4
Outdoor	Ambient	-1.2	14.0	15.2	6.1	±3.4
	1o rectum	-0.7	12.8	13.5	5.8	±3.2

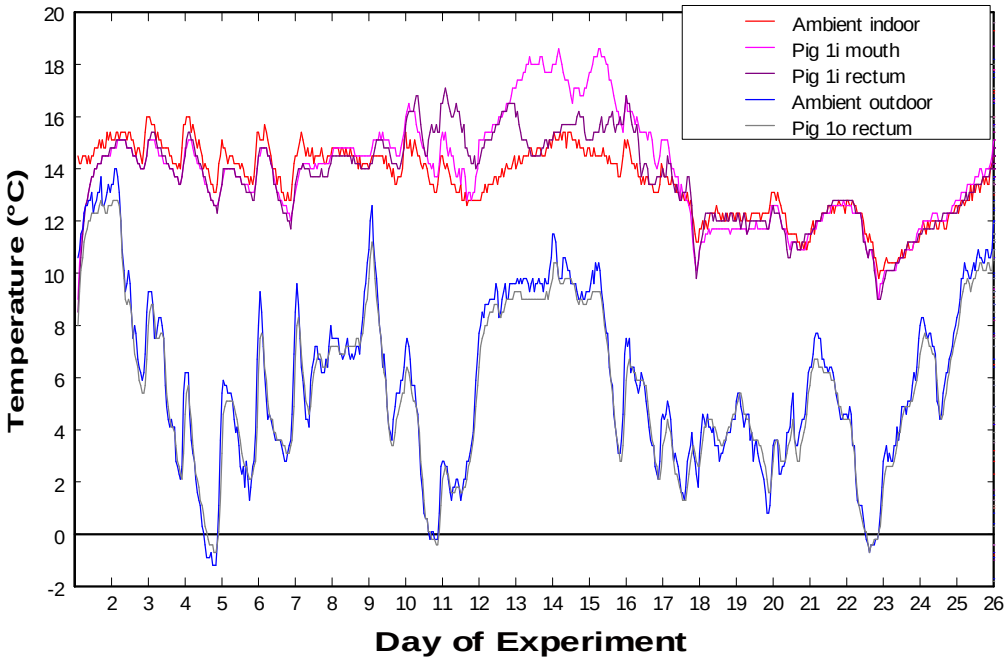


Figure 3.2.3.4 Run 1: datalogger recordings of ambient indoor and outdoor temperatures and datalogger probe temperatures in the indoor pig mouth and rectum, and in the outdoor pig rectum from Day 1-26.

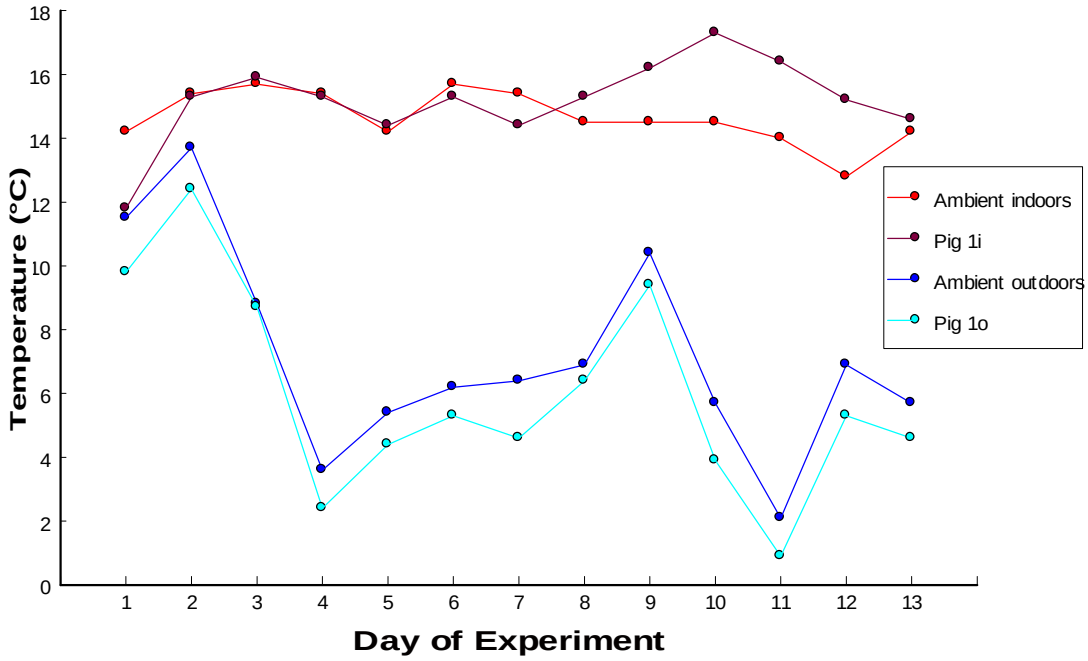


Figure 3.2.3.5 Run 1: infrared temperature recordings measured at each visit of the highest larval mass temperatures on the indoor pig and the outdoor pig, and ambient temperature measured at the same time using digital probe datalogger, from Day 1-13.

Table 3.2.3.4 Run 1: indoor pig (1i) observation of decomposition and larval stages

Day of Exp:	1	2	3	4	5	6	7	8	9	10	11	12	16	28	29	31	32	35	39	40	42	44	46	55	56	61	63	66	76	81
Decomp																														
Egg masses																														
1st instar																														
2nd instar																														
3rd instar																														
Wandering																														
Pupae																														
Eclosion																														

Table 3.2.3.5 Run 1: outdoor pig (1o) observation of decomposition and larval stages

Day of Exp:	1	2	3	4	5	6	7	8	9	10	11	12	16	28	29	31	32	35	39	40	42	44	46	55	56	61	63	66	76	81
Decomp																														
Egg masses																														
1st instar																														
2nd instar																														
3rd instar																														
Wandering																														
Pupae																														
Eclosion																														

Key to decomposition stages:	Fresh	Bloat	Active decay	Dry decay	Skeletal

3.2.3.2 Run 2

Table 3.2.3.6 Run 2: Field observations recorded at each visit to indoor and outdoor piglets

Day	Date & time	Weather	Piglet 2i (indoor)	Piglet 2o (outdoor)
1	27th Feb 2004 12:00 [started rectal dataloggers]	[no comment]	[no comment]	[no comment]
3	29th Feb 2004 17:00	[no comment]	No maggot masses	Small maggot mass in mouth
4	1st March 2004 16:00	W0/R0/C1	1 Calliphorid on snout; no eggs	Just a few eggs under hind legs
5	2nd March 2004	W0/R0/C2	Flies seen; no masses	No maggot masses
6	3rd March 2004	W1/R0/C3	No maggot masses	No maggot masses
7	4th March 2004	W0/R1/C3	No maggot masses	No maggot masses
8	5th March 2004	W0/R0/C3	No maggot masses	No maggot masses; Calliphora under back of neck
9	6th March 2004	W0/R1/C2	No maggot masses	No maggot masses
11	8th March 2004	W1/R0/C1	2 Calliphora in room; no maggot masses	No maggot masses
13	10th March 2004	W1/R0/C2	Tiny egg masses between back legs	[No comment]
14	11th March 2004	W2/R0/C3	[No comment]	[No comment]
15	12th March 2004	W0/R2/C3	1st instars on snout / bowl	[No comment]
16	13th March 2004	W3/C1/R0	2 Calliphora on stomach; blood under head	[No comment]
18	15th March 2004 [dataloggers put in mouth of both piglets]	W2/R1/C3	Large maggot mass in mouth and in stomach	Egg mass on neck
19	16th March 2004	W2/R0/C0	Maggot mass in neck as well	2 Calliphora flying around
20	17th March 2004	W1/R0/C3	Calliphora on body	Calliphora seen at body
21	18th March 2004	W3/R0/C3	[No comment]	[No comment]
22	19th March 2004	W2/R1/C2	[No comment]	Cheek is swollen up
23	20th March 2004	W3/R1/C3	Adult Calliphora on body; first puparia in sand	[No comment]
24	21st March 2004 18:15	W2/R0/C2	[No comment]	Turned over due to high winds; both probes out; lots of 3rd instars
25	22nd March 2004	W1/R0/C1	[No comment]	[No comment]
26	23rd March 2004	W2/R1/C2	[No comment]	2 Calliphora on piglet
27	24th March 2004	W2/R1/C1	[No comment]	[No comment]
28	25th March 2004	W2/R0/C1	[No comment]	2 Calliphora
29	26th March 2004	[no comment]	[No comment]	Calliphora present; maggot mass on neck
34	31st March 2004	W1/R0/C0	[No comment]	[No comment]
35	1st April 2004	W2/R0/C1	Puparia put in cages/fridge	Put in sandbox; a few pupae
47	13th April 2004	W1/R0/C2	Adults emerged from puparia in cages [date unknown]; some still emerging and very little sugar consumed therefore 2/3 days earlier?	Lots of dark puparia; wandering larvae under box
48	14th April 2004	W1/R0/C0	More puparia collected	[No comment]
50	16th April 2004	W1/R0/C0	[No comment]	[No comment]
60	25th April 2004	[No comment]	Puparia from fridge emerged	[No comment]
66	1st May 2004	[No comment]	[No comment]	1st batch of puparia now emerged

Figure 3.2.3.6 Photographs taken during decomposition of indoor (2i) and outdoor (2o) piglets in Run 2, stating day and date of experiment

Table 3.2.3.7 Run 2 datalogger statistics (Ambient & Rectum: Day 1 12:00 to Day 50 12:00; Mouth: Day 18 17:00 to Day 50 12:00)

Run 2		Min (°C)	Max (°C)	Range (°C)	Mean (°C)	SD
Indoor	Ambient	6.2	29.1	22.9	13.2	±2.6
	2i mouth	18.0	20.2	2.2	19.1	±1.6
	2i rectum	-1.9	39.9	41.8	13.9	±3.3
Outdoor	Ambient	4.1	20.2	16.1	12.2	±11.4
	2o mouth	12.0	18.0	6.0	15.0	±4.2
	2o rectum	0.8	18.9	18.1	9.9	±12.8

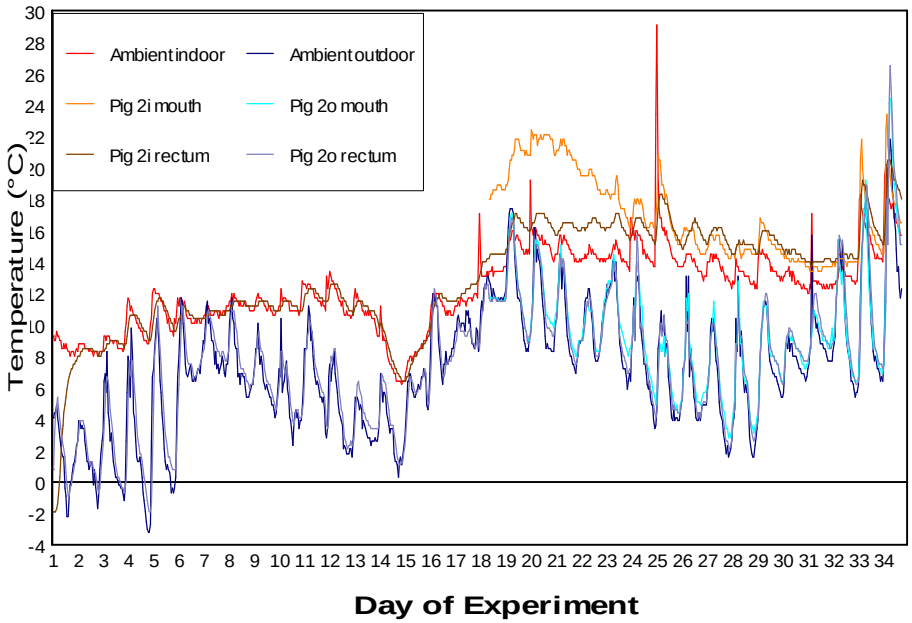


Figure 3.2.3.7 Run 2: datalogger recordings of ambient indoor and outdoor temperatures and datalogger probe temperatures in the indoor and outdoor pig mouth and rectum, from Day 1-34.

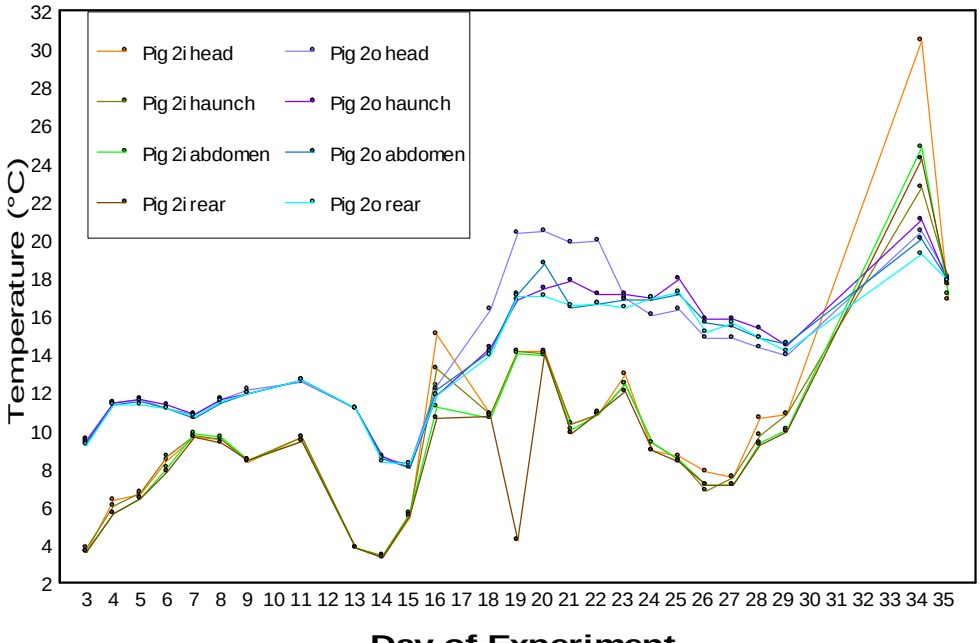


Figure 3.2.3.8 Run 2: infrared temperature recordings measured at each visit of the highest larval mass temperatures on the indoor pig and the outdoor pig, and ambient temperature measured at the same time using digital probe datalogger from Day 1-35.

Table 3.2.3.8 Run 2: indoor pig (2i) observation of decomposition and larval stages

Day of Expt:	1	3	4	5	6	7	8	9	11	13	14	15	16	18	19	20	21	22	23	24	25	26	27	28	29	34	35	47	48	50	60	66
Decomp																																
Egg masses																																
1st instar																																
2nd instar																																
3rd instar																																
Wandering																																
Pupae																																
Eclosion																																

Table 3.2.3.9 Run 2: outdoor pig (2i) observation of decomposition and larval stages

Day of Expt:	1	3	4	5	6	7	8	9	11	13	14	15	16	18	19	20	21	22	23	24	25	26	27	28	29	34	35	47	48	50	60	66
Decomp																																
Egg masses																																
1st instar																																
2nd instar																																
3rd instar																																
Wandering																																
Pupae																																
Eclosion																																

Key to decomposition stages:	Fresh	Bloat	Active decay	Dry decay	Skeletal

3.2.3.3 Run 3

Table 3.2.3.10 Run 3: Field observations recorded at each visit to indoor and outdoor piglets

Day	Date & time	Weather	Piglet 3i (indoor)	Piglet 3o (outdoor)
1	22nd May 2004 [started dataloggers]	[no comment]	[no comment]	Flies on it within minutes
2	23rd May 2004 18:30	W1/R0/C1	Bloated; Calliphora on head and under body; eggs laid between front legs, in ear, on left knee	Calliphorids on body & sticky paper ; big egg masses between back legs, front legs, behind right ear & under body
3	24th May 2004 17:15	W0/R0/C2	Completely bloated & marbled; lots of blood; lots of Calliphora	Calliphorids + 5 on flypaper; egg mass hatched; 1st instars sampled
4	25th May 2004 18:00	W1/R0/C2	Lots of Calliphora, approx. 11 at any one time	Bloated; Calliphora only, no Lucilia
5	26th May 2004 18:00	W1/R0/C1	Abdominal bloating gone down; maggot masses in abdomen, mouth & back legs	Only a few flies; grey & bloated; bloody underneath; maggot masses in left fore-armpit; 1 Lucilia; maggot mass under skin in front of right ear
6	27th May 2004 19:00	W1/R0/C1	Head completely gone, as is abdomen; only a few flies, mainly in room, not on piglet	Just a few Calliphora; big hole behind ear; very dry, black inside
7	28th May 2004 17:20	W2/R0/C1	Quite a lot of Calliphora around; not all on body, but in room and on side of bowl; head skinless; large maggot mass in neck, rear, back & spine exposed	Grey & gooey; all maggots inside body, not visible from outside; just 1 or 2 Calliphora; 1 Lucilia seen in the morning
8	29th May 2004 18:30	W1/R0/C2	Maggots still leaving the body; no soft tissues left, therefore quite small; dead adult flies being fed on by larvae	Spine exposed; face puffed up; Calliphorids
10	31st May 2004 17:00	W2/R0/C1	[no comment]	Wandering maggots; 1st day that Sarcophagids are seen; body totally flattened; large numbers of larvae under bowl; piglet and maggots put into sand bowl
11	1st June 2004	[no comment]	Still 3rd instar wandering larvae; a few puparia	Still 3rd instar wandering larvae
12	2nd June 2004	[no comment]	Puparia present	Puparia present
17	7th June 2004 12:00	[no comment]	Puparia collected and put in cages	Puparia collected and put in cages
24	14th June 2004	[no comment]	Puparia have emerged as flies	Puparia have emerged as flies
28	18th June 2004	[no comment]	Tiny flies have emerged; small flies on body	All puparia have emerged as flies

Figure 3.2.3.9 Photographs taken during decomposition of indoor (3i) and outdoor (3o) piglets in Run 3, stating day and date of experiment

Table 3.2.3.11 Replicate 3 datalogger statistics (Day 1 14:00 to Day 17 20:00)

Run 3		Min (°C)	Max (°C)	Range (°C)	Mean (°C)	SD
Indoor	Ambient	16.8	31.4	14.6	22.0	±2.3
	3i mouth	17.1	31.0	13.9	22.2	±2.5
	3i rectum	17.4	33.0	15.6	22.0	±2.4
Outdoor	Ambient	6.7	32.2	25.5	17.7	±0.4
	3o mouth	7.7	31.8	24.1	18.7	±1.6
	3o rectum	8.0	37.0	29.0	17.9	±4.8

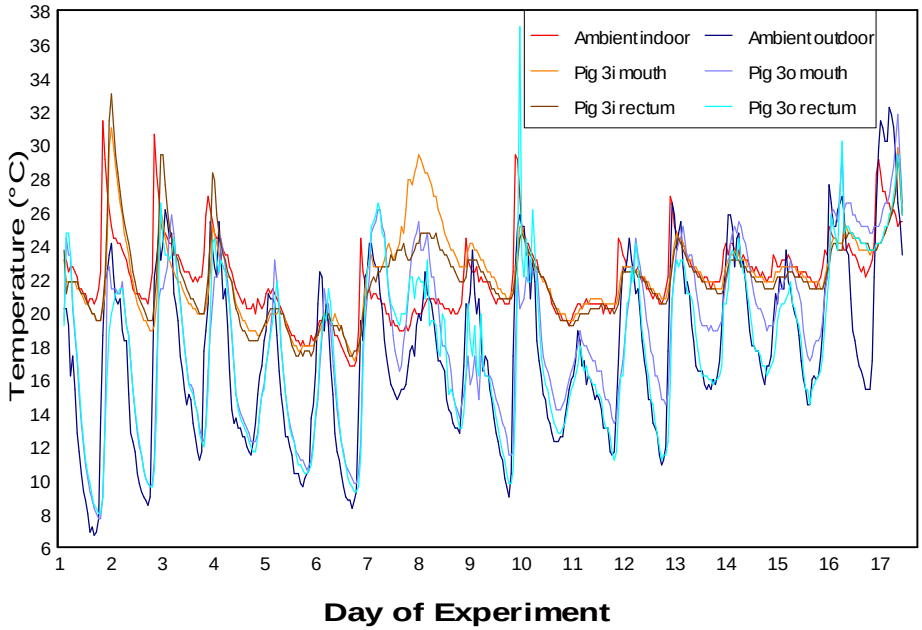


Figure 3.2.3.10 Run 3: datalogger recordings of ambient indoor and outdoor temperatures and datalogger probe temperatures in the indoor and outdoor pig mouth and rectum, from Day 1-17.

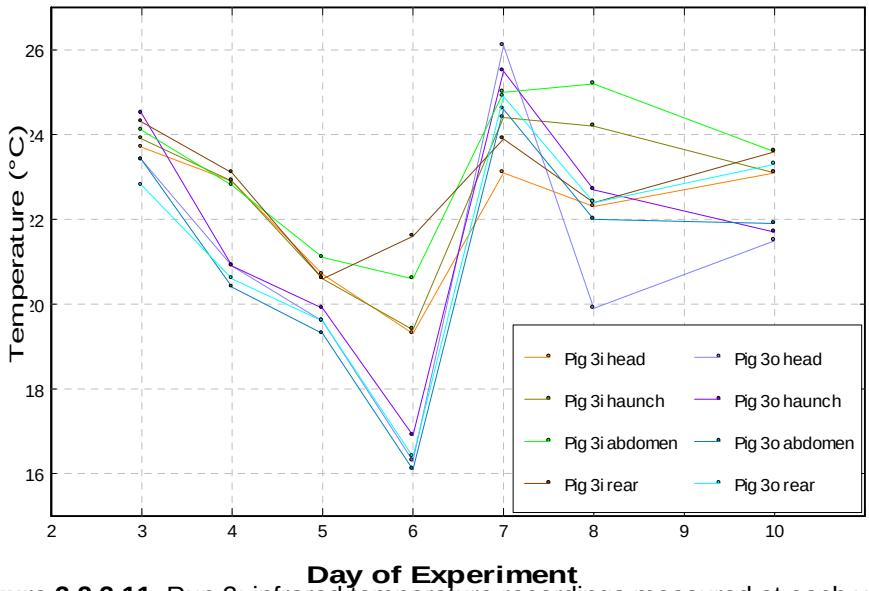


Figure 3.2.3.11 Run 3: infrared temperature recordings measured at each visit of the highest larval mass temperatures on the indoor pig and the outdoor pig, and ambient temperature measured at the same time using digital probe datalogger from Day 3-10.

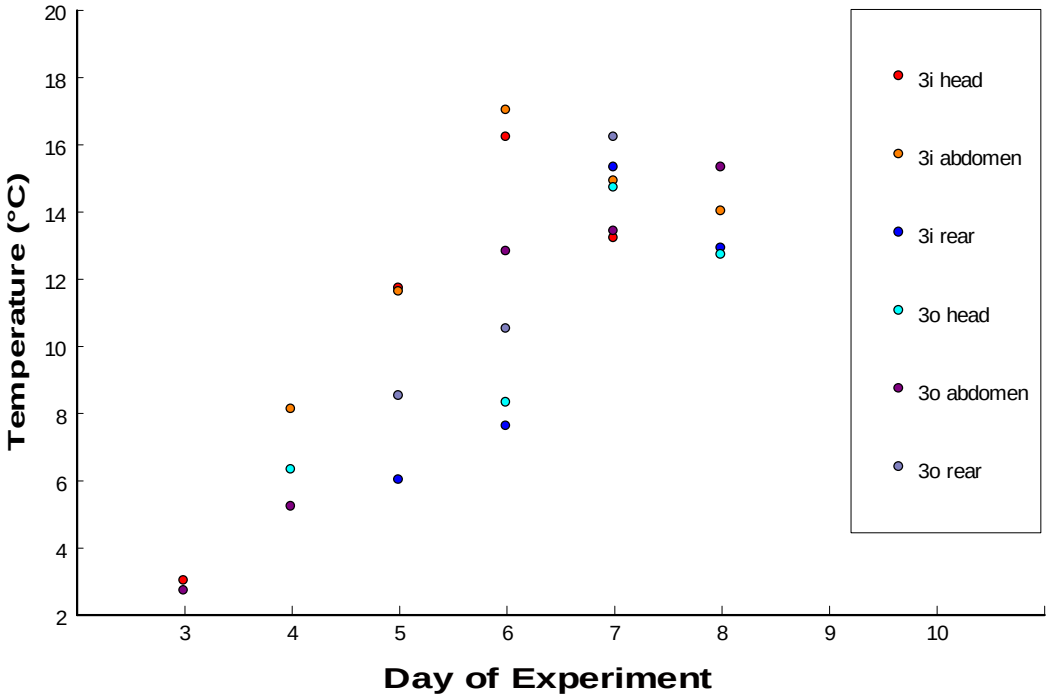


Figure 3.2.3.12 Run 3: mean lengths of *Calliphora vicina* larvae collected from indoor and outdoor piglets

Table 3.2.3.12 Run 3: indoor pig (3i) observation of decomposition and larval stages

Day of Exp:	1	2	3	4	5	6	7	8	10	11	12	17	24	28
Decomp														
Egg masses														
1st instar														
2nd instar														
3rd instar														
Wandering														
Pupae														
Eclosion														

Table 3.2.3.13 Run 3: outdoor pig (3o) observation of decomposition and larval stages

Day of Exp:	1	2	3	4	5	6	7	8	10	11	12	17	24	28
Decomp														
Egg masses														
1st instar														
2nd instar														
3rd instar														
Wandering														
Pupae														
Eclosion														

Key to decomposition stages:				
Fresh	**Bloat**	**Active decay**	**Dry decay**	**Skeletal**

3.2.3.4 Run 4

Table 3.2.3.14 Run 4: Field observations recorded at each visit to indoor and outdoor piglets

Day	Date & time	Weather	Piglet 4i (indoor)	Piglet 4o (outdoor)
1	2nd March 2005 [started dataloggers]	[no comment]	[no comment]	[no comment]
2	3rd March 2005 15:30	W2/R0/C1	No eggs or flies (too cold)	No eggs or flies (too cold)
6	7th March 2005 16:30	W0/R0/C2	No eggs/flies, still quite cold; some fluid from noses & mouths	No eggs/flies, still quite cold; some fluid from noses & mouths
7	8th March 2005 17:00	W1/R0/C3	1 Calliphorid on back leg; eggs laid between back legs	No eggs or flies
8	9th March 2005 15:30	W0/R0/C3	1 Calliphorid laying eggs; no hatched larvae	No eggs or flies
9	10th March 2005 16:00	W0/R0/C1	1 Calliphorid laying on inside back legs; one egg mass under bottom back leg; nose & mouth bloody but not attracting flies	1 Calliphorid on piglet, but no egg masses
10	11th March 2005	W2/R0/C3	1 Calliphora; bloating has started; 1st egg masses have hatched; egg mass laid yesterday not yet hatched (48 hours?)	No eggs or flies
11	12th March 2005 16:00	W3/R0/C2	Beginning to smell; bloated; 1 Calliphorid; new egg mass under head/neck area	No eggs or flies
13	14th March 2005 16:30	W2/C0/R0	Very smelly and bloody; still bloated; maggot mass in belly; egg mass under leg hatched but dispersed as too wet there; new egg mass under thigh	No eggs or flies
14	15th March 2005 16:30	W2/R0/C2	Very bloody; big hole in groin area; 2 flies in room; new egg masses under front legs and in mouth; maggot mass in belly button	Still nothing
15	16th March 2005 17:30	W2/R1/C3	New egg mass under chin	No flies or eggs
16	17th March 2005 17:30	W2/C0/R0	Flies present; more egg masses down front legs, between front legs and under tongue	First flies seen on piglet; small egg masses on back/bowl, under front legs, under back legs; bloating
17	18th March 2005 17:00	W2/C0/R0	Lots more flies in room; lots of flies still laying in ear and shoulder	New egg masses; nothing hatched; 6+ Calliphora on piglet; in bloat
20	21st March 2005 15:30	W1/C3/R0	No flies on piglet but 2-3 in room; fresh egg masses around rectum; maggot masses between rear legs, in stomach and mouth	1st instar larvae between front legs; eggs between rear legs; several flies on piglet
21	22nd March 2005 14:30	W2/C3/R1	Stomach & head maggot masses gone except for individuals; large maggot mass at rear end; migrating larvae in sandbox; flies in room	New egg masses and 1st instar larvae between back legs; new eggs on chin and upper neck; several flies on piglet

22	23rd March 2005 16:30	W1/C2/R0	Almost a full skeleton; first puparia seen in sand; maggot mass one end of rear leg	More smelly; flies laying eggs under rear limb; larvae under head and between rear legs, and in lower abdomen
23	24th March 2005 17:30	W2/C1/R1	Larvae grouped under spine; lots of dark puparia in sand; lots of small larvae in bowl that have moved away from food source	No larvae visible from undisturbed view; more larvae between rear legs and under stomach than under head
26	27th March 2005 11:30	W1/R0/C3	Lots of puparia and some post-feeding 3rd instars; body virtually skeletonised	Egg masses front & back; slow-moving 3rd instars
28	29th March 2005 16:30	W1/R0/C3	Lots of puparia and post-feeding larvae inside carcass; most puparia collected and put into fly cages	Maggot mass throughout body, but not visible without lifting up body; small 3rd instars under bowl; put into sandbox
29	30th March 2005 16:30	W1/R1/C3	Still some small 3rd instars in sand; no puparia have emerged yet	Larvae very sluggish, under body mainly, no puparia yet
31	1st April 2005 11:30	W1/R0/C1	Still some 3rd instars under bowl; puparia not emerged	No puparia yet, but some post-feeding larvae, many staying under body instead of moving off
34	4th April 2005 16:30	[no comment]	Still a few post-feeding 3rd instars under carcass; no emerged puparia	Lots of post-feeding 3rds under carcass, not moving away from body, but finished feeding; some small, pale puparia in sand (day before?)
41	11th April 2005	[no comment	Adults emerged from puparia – wings formed but little frass in cage, therefore emerged previous day?	[no comment]
44	14th April 2005 [dataloggers removed]	[no comment]	[no comment]	No emerged puparia
55	25th April 2005 17:30	[no comment]	[no comment]	No emerged puparia
58	28th April 2005	[no comment]	[no comment]	No emerged puparia
63	3rd May 2005 14:00	[no comment]	[no comment]	Lots of flies, including dead ones

Figure 3.2.3.13 Photographs taken during decomposition of indoor (4i) and outdoor (4o) piglets in Run 4, stating day and date of experiment

Table 3.2.3.15 Run 4 datalogger statistics (Day 1 16:00 to Day 45 16:00)

Run 4		Min (°C)	Max (°C)	Range (°C)	Mean (°C)	SD
Indoor	Ambient	8.8	35.7	26.9	15.0	±3.2
	4i mouth	10.9	46.0	35.1	25.8	±6.0
	4i rectum	8.8	24.7	15.9	14.9	±3.0
Outdoor	Ambient	-2.7	25.1	27.8	9.5	±4.9
	4o mouth	-2.7	25.8	28.5	9.8	±5.3
	4o rectum	-2.5	27.2	29.7	10.0	±5.3

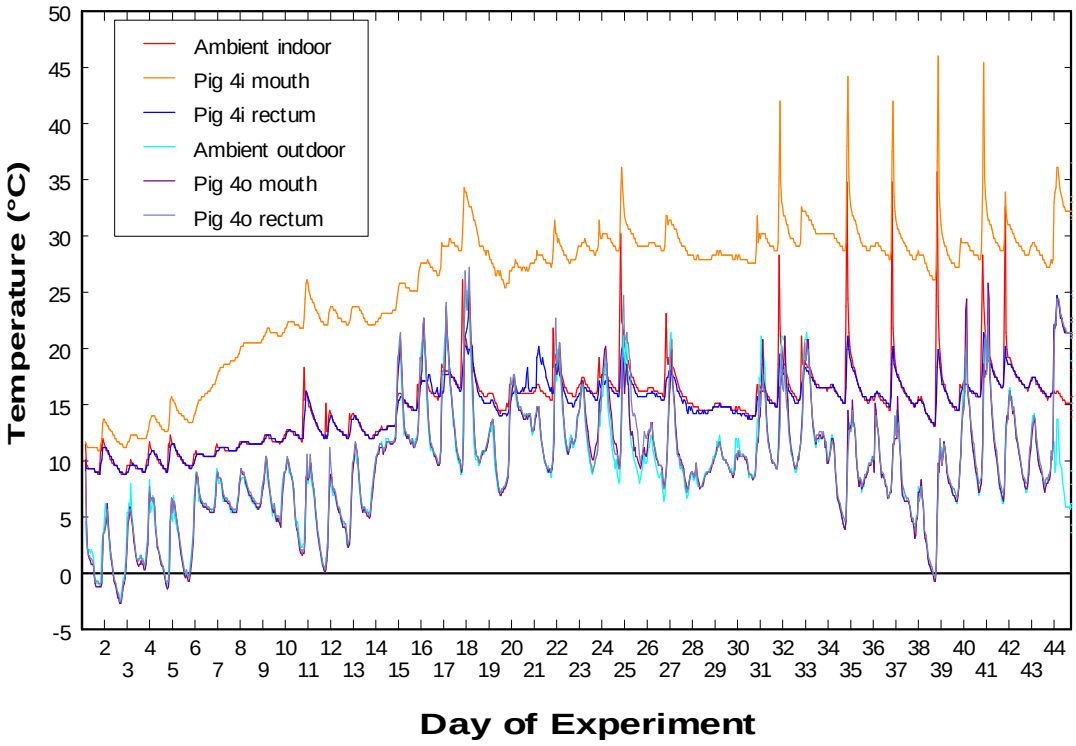


Figure 3.2.3.14 Run 4: datalogger recordings of ambient indoor and outdoor temperatures and datalogger probe temperatures in the indoor and outdoor pig mouth and rectum, from Day 1-44.

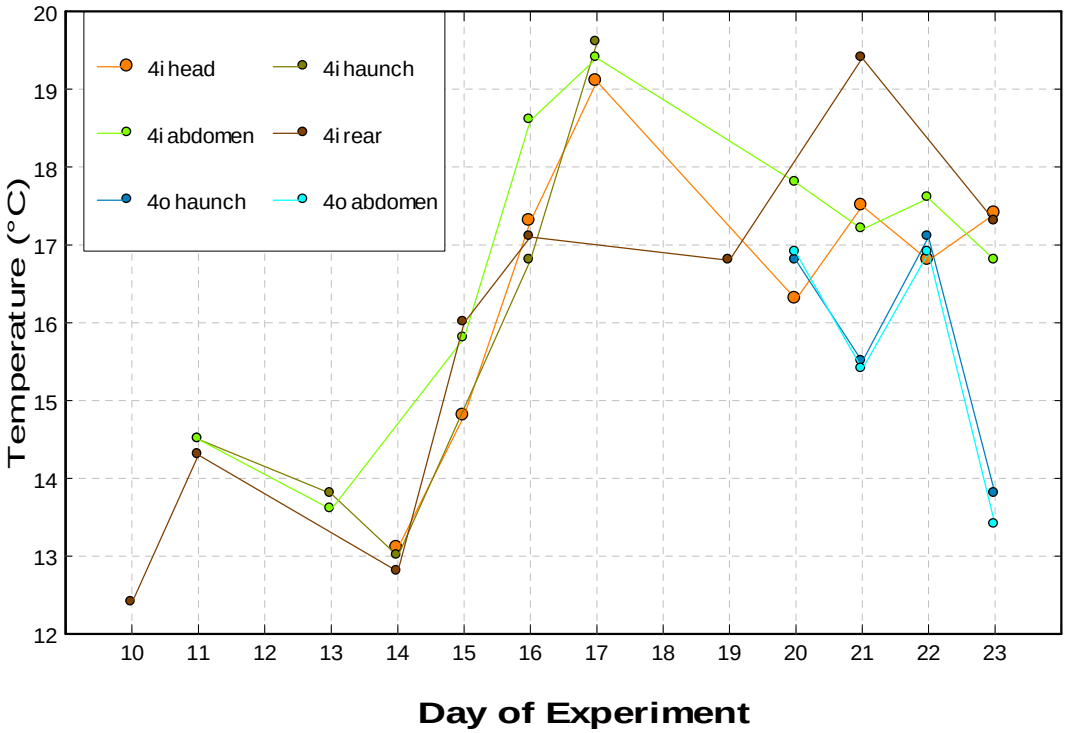


Figure 3.2.3.15 Run 4: infrared temperature recordings measured at each visit of the highest larval mass temperatures on the indoor pig and the outdoor pig, and ambient temperature measured at the same time using digital probe datalogger from Day 10-2

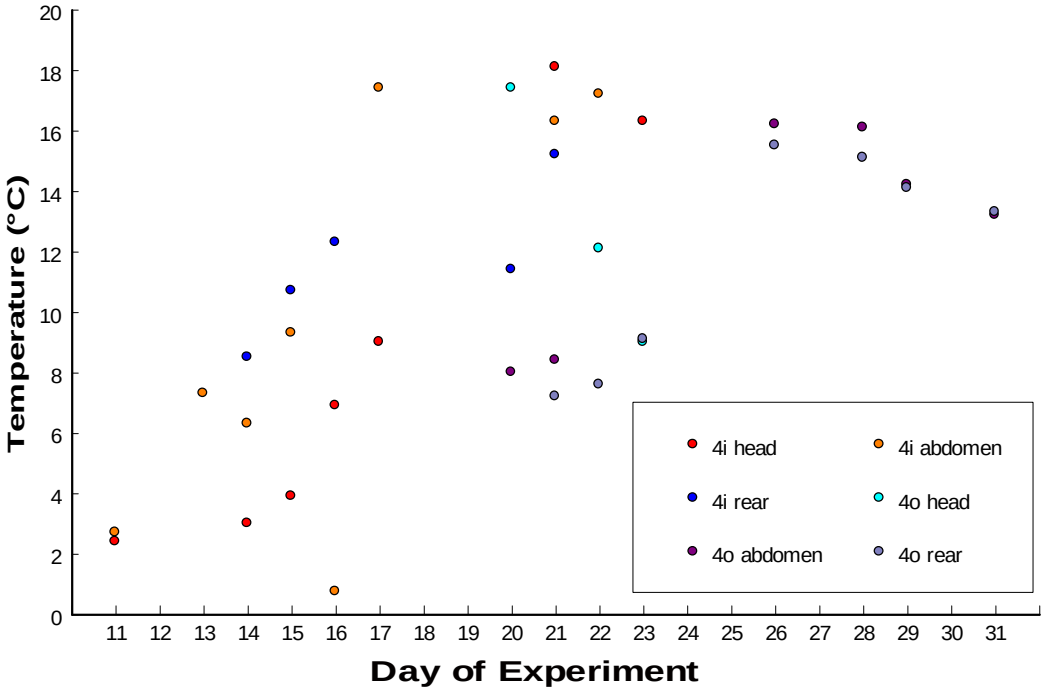


Figure 3.2.3.16 Run 4: mean lengths of *Calliphora vicina* larvae collected from indoor and outdoor piglets

Table 3.2.3.16 Run 4: indoor pig (4i) observation of decomposition and larval stages

Day of Exp:	1	2	6	7	8	9	10	11	13	14	15	16	17	20	21	22	23	26	28	29	31	34	41	44	55	58	63
Decomp																											
Egg masses																											
1st instar																											
2nd instar																											
3rd instar																											
Wandering																											
Pupae																											
Eclosion																											

Table 3.2.3.17 Run 4: outdoor pig (4o) observation of decomposition and larval stages

Day of Exp:	1	2	6	7	8	9	10	11	13	14	15	16	17	20	21	22	23	26	28	29	31	34	41	44	55	58	63
Decomp																											
Egg masses																											
1st instar																											
2nd instar																											
3rd instar																											
Wandering																											
Pupae																											
Eclosion																											

Key to decomposition stages:	Fresh	Bloat	Active decay	Dry decay	Skeletal

3.2.3.5 Run 5

Table 3.2.3.18 Run 5: Field observations recorded at each visit to indoor and outdoor piglets

Day	Date & time	Weather	Piglet 5i (indoor)	Piglet 5o (outdoor)
1	2nd August 2005 [started dataloggers]	[no comment]	1 Muscid in room	[no comment]
2	3rd August 2005 15:15	[no comment]	No flies seen; no marbling, bloating or egg masses	2 Lucilia and 2 Sarcophagids this morning; small egg masses under front legs and head
3	4th August 2005 15:00	W2/R0/C0	No flies or eggs	Small egg masses under front and back legs (new); eggs under head have hatched into 1st instars
4	5th August 2005 17:00	W2/R2/C2	2 Calliphora; small larvae under head; eggs between front legs	3 wasps; 1 Sarcophagid; egg masses on abdomen; 1st instars under head and legs; 2nd instars behind ear
5	6th August 2005 17:00	W2/R0/C1	Full bloat; 5 Calliphora; larvae in mouth, lots of blood	Wasps in belly button; lots of blood; full bloat; 2 Calliphids; egg on stomach
6	7th August 2005 17:00	W2/R0/C2	Very bloated & red; fresh egg masses under chin and on legs; 3 Calliphora, 1 Lucilia	Bloated; wasps; new eggs on snout and under belly; 1 Sarco & 1 Calliphora
7	8th August 2005 18:00	W0/R0/C1	Still flies and in bloat	Wasps
8	9th August 2005 17:00	W0/R0/C1	Still bloated and bloody; large larvae in blood; fresh egg masses down back	Still wasps; hole in belly button; larvae moved down to neck area; new egg mass on shoulder
9	10th August 2005 17:30	[no comment]	Large maggot mass in abdomen; larvae have started leaving the body; 2-3 cohorts in abdomen	No maggots visible, all under body; 2 cohorts present; have started to leave body; put in sawdust box
10	11th August 2005 19:00	W1/R0/C2	Puparia in sand; still some larvae in body cavity; smaller larvae in abdomen	Lots of larvae in sawdust; no puparia yet; only 1 wasp and no longer feeding on body
11	12th August 2005 11:30	W2/R0/C2	22 puparia put into incubator set at 25C with mouth probe; puparia in sandbox; still some larvae on body; sarcos present	Still a few wasps; 2 sizes of larvae on body; rectal probe replaced
12	13th August 2005 18:00	W2/R2/C3	Dark puparia in sand; sarcos in bowl still moving around	Still some maggots under and in body; a few puparia in sawdust
13	14th August 2005 17:00	W1/R1/C2	Lots of post-feeding wandering larvae in bowl; lots also under body; tiny parasitoids	Some puparia in sandbox; lots of post-feeding larvae; some small larvae under/inside body; 1 wasp
14	15th August 2005 17:15	W0/R0/C1	Puparia put in cage; post-feeding Sarcophagid larvae	Still post-feeding larvae under body; some puparia
15	16th August 2005 17:00 [dataloggers removed]	W0/R0/C0	Lots more puparia to collect; no flies emerged	Puparia put in cage; still lots of pre-pupae
31	1st September 2005	[no comment]	Lots of flies in cages	Only 1 dead *Lucilia*; no pupae emerged
37	6th September 2005	[no comment]	Still lots of flies	Lots of parasitic wasps emerged from puparia

Figure 3.2.3.17 Photographs taken during decomposition of indoor (5i) and outdoor (5o) piglets in Run 5, stating day and date of experiment

Table 3.2.3.19 Run 5 datalogger statistics (Day 1 16:00 to Day 15 17:00 (except to Day 11 12:00 for 5i mouth datalogger)

Run 5		Min (°C)	Max (°C)	Range (°C)	Mean (°C)	SD
Indoor	Ambient	20.2	30.2	10.0	22.7	±1.6
	5i mouth	20.2	34.3	14.1	22.9	±2.0
	5i rectum	24.1	43.7	19.6	30.1	±4.3
Outdoor	Ambient	10.9	31.4	20.5	18.9	±4.5
	5o mouth	11.5	35.2	23.7	19.0	±4.4
	5o rectum	11.2	38.5	27.3	19.3	±5.0

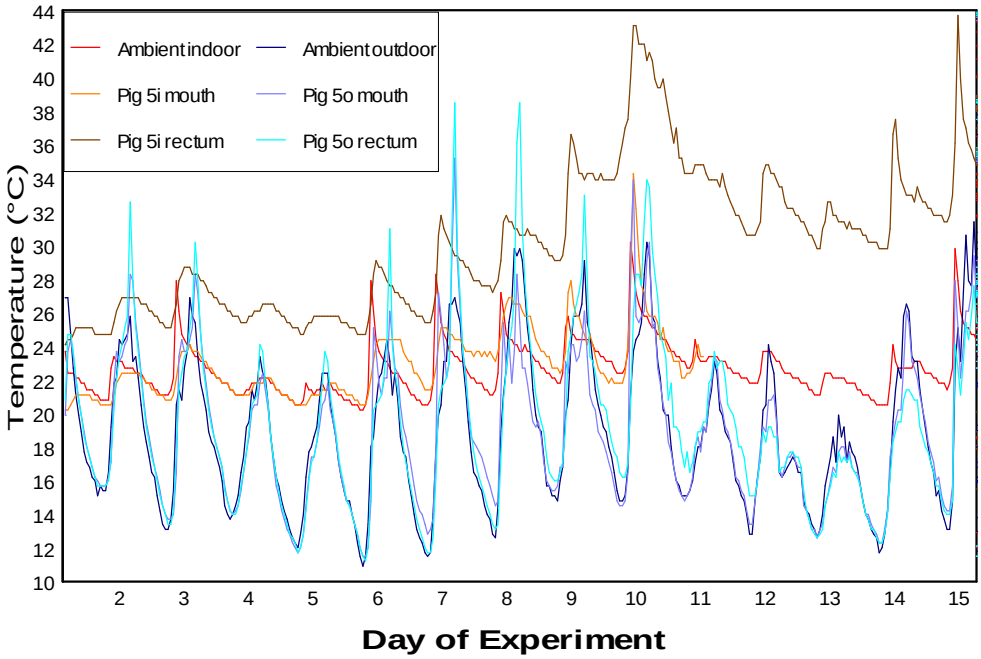


Figure 3.2.3.18 Run 5: datalogger recordings of ambient indoor and outdoor temperatures and datalogger probe temperatures in the indoor and outdoor pig mouth and rectum, from Day 1-34.

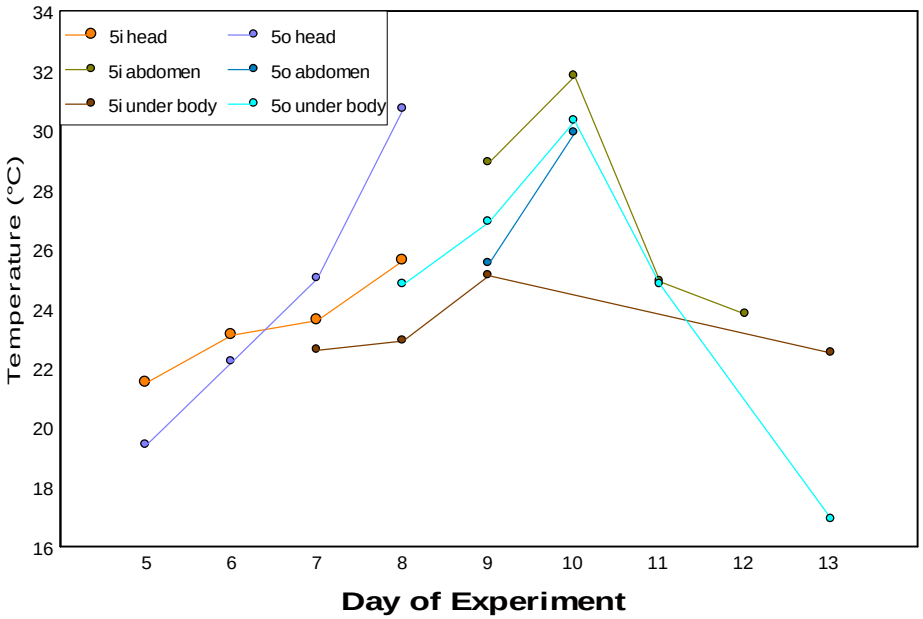


Figure 3.2.3.19 Run 5: infrared temperature recordings measured at each visit of the highest larval mass temperatures on the indoor pig and the outdoor pig, and ambient temperature measured at the same time using digital probe datalogger from Day 5-13.

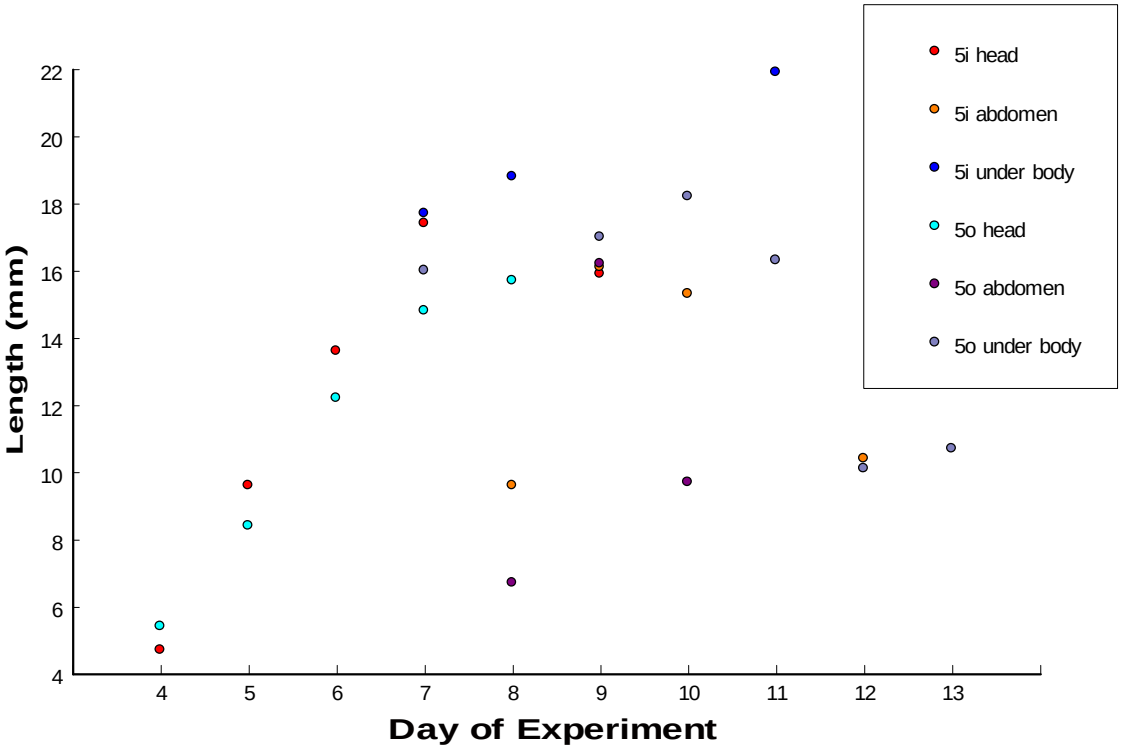


Figure 3.2.3.20 Run 5: mean lengths of *Calliphora vicina* larvae collected from indoor and outdoor piglets

Table 3.2.3.20 Run 5: indoor pig (5i) observation of decomposition and larval stages

Day of Exp:	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	31	37	79
Decomp																		
Egg masses																		
1st instar																		
2nd instar																		
3rd instar																		
Wandering																		
Pupae																		
Eclosion																		

Table 3.2.3.21 Run 5: outdoor pig (5o) observation of decomposition and larval stages

Pig 4o	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	31	37	79
Decomp																		
Egg masses																		
1st instar																		
2nd instar																		
3rd instar																		
Wandering																		
Pupae																		
Eclosion																		

Key to decomposition stages:				
Fresh	**Bloat**	**Active decay**	**Dry decay**	**Skeletal**

3.2.3.6 Combined results

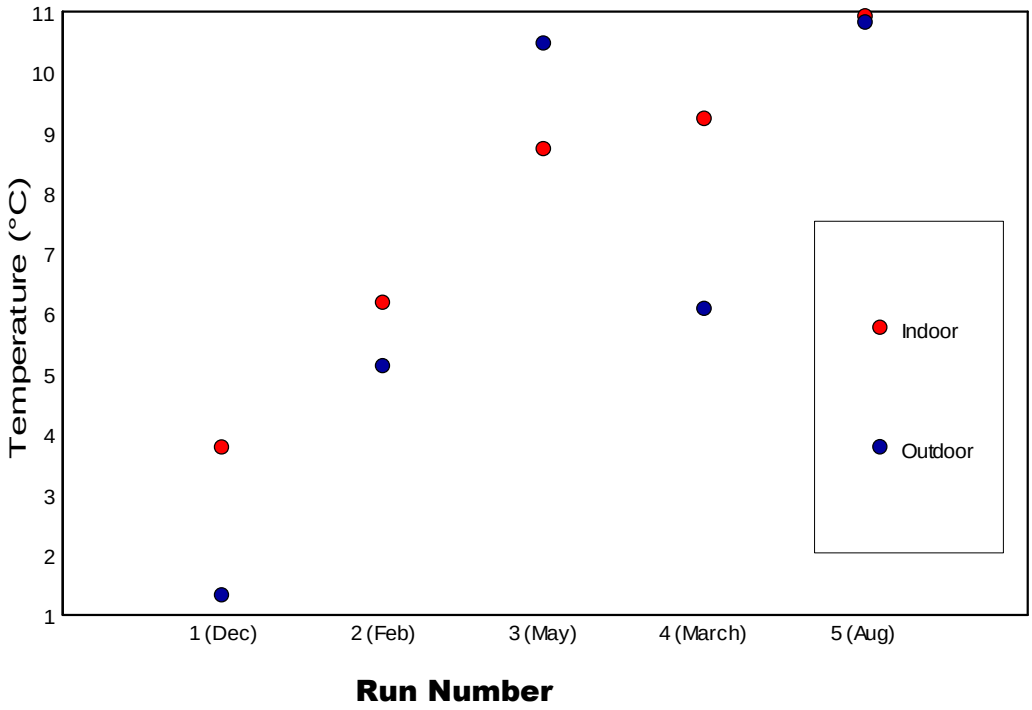


Figure 3.2.3.21 Maximum datalogger temperature above ambient, ordered chronologically by run start date: 1 (December 2003), 2 (February 2004), 3 (May 2004), 4 (March 2005), 5 (August 2005).

The increase in larval mass temperatures above the ambient temperature were measured by datalogger probes and the maximum difference in temperature for each cadaver are shown in Figure 3.2.3.21. At colder ambient temperatures, e.g. in Run 1, the increase above ambient is relatively small, and only just above 1°C in the outdoor piglet. As the ambient temperature increases, however, the larval mass temperatures also increase, so during the hottest period, in Run 5, the highest difference between larval mass temperatures and ambient temperatures are 11°C. In addition, there is very little difference between the indoor and outdoor maximum temperature above ambient.

Table 3.2.3.22 List of piglets [i=indoor; o=outdoor] and the Accumulated Degree Hours (ADH) from first egg-laying to first emergence of adults for Runs 1-5

Run No.	Piglet	Datalogger	ADH
1	1i	Ambient Mouth Rectum	10901.5 11184.0 11002.9
	1o	Ambient Rectum	8111.5 7912.0
2	2i	Ambient Mouth Rectum	10561.3 11785.8 11485.4
	2o	Ambient Mouth Rectum	12817.1 12808.4 12783.4
3	3i	Ambient Mouth Rectum	11555.6 11634.7 11537.2
	3o	Ambient Mouth Rectum	9891.6 10299.9 9995.4
4	4i	Ambient Mouth Rectum	12007.3 21647.5 11692.0
	4o	Ambient Mouth Rectum	12248.2 12668.4 12790.9
5	5i	Ambient Mouth Rectum	14394.9 14730.7 17414.2
	5o	Ambient Mouth Rectum	No emergence of adult flies due to parasitism

Table 3.2.3.22 shows the ADH (Accumulated Degree Hours), using a base temperature of 1°C, calculated for each datalogger (ambient, mouth and rectal) from the time the first eggs were seen on the body to the first emergence of adults. The mouth datalogger for piglet 4i shows a much higher ADH, which is clearly outside the normal range, perhaps caused by sunlight falling directly onto the probe. The ADH's for piglet 5i are also higher, due to the fact that there was a 15 day delay between checks on the piglet, during which time the adults emerged, therefore these estimates are most likely longer than the true PMI. In addition, the majority of pupae from piglet 5o were parasitized and therefore did not emerge as adults. The mean ADH for the four indoor piglets is

11433.112 and for the four outdoor piglets is 10861.545, a little above the range of 9550-10550 suggested by Amendt *et al*. (2007).

Calliphora vicina was present on all cadavers, and was dominant in runs 1 and 4. *Lucilia sericata* was also present in runs 2, 3 and 5 though in smaller numbers. A small number of Sarcophagids were seen on the outdoor piglets in runs 3 and 5. The outdoor piglet in run 5 attracted a great number of wasps, and almost all the Calliphorid pupae were parasitized by the wasp species *Nasonia vitripennis* Walker 1836 and *Alysia manducator* Panzer 1799. The indoor piglets in runs 2 and 3 were also infested with *Dermestes* spp. Linnaeus 1758. Neither parasitoids nor cadaverous beetle species prevented pupation in the blowfly species.

During two out of the five runs, the outdoor ambient temperature dropped below freezing, to a minimum of -1.2°C in run 1 and -2.7°C in run 4. The highest outdoor ambient temperature reached was 32.2°C in run 3.

In runs 1 and 3, oviposition was observed on the same day in both the indoor and outdoor piglets, Day 3 in run 1 and Day 2 in run 3. Larval development continued at a similar rate in run 3 (Tables 3.2.3.12 & 3.2.313) and the photographs in Figure 3.2.3.8 indicate that the rate of decomposition was only a little delayed in the outdoor piglet. Although the maximum ambient temperatures were similar (32.2°C outdoors and 31.4°C indoors), the minimum indoor temperatures were 10°C higher than those outdoors, 16°C as opposed to 6.7°C. In contrast, in run 1, although the eggs were laid on Day 3 on both piglets, the temperature outdoors dropped at the same time, reaching below freezing on Day 5. Therefore the egg masses on the outdoor piglet did not begin to hatch until Day 12. As a result of this, the decomposition of the piglet was dramatically reduced, as can be seen in the photographs (Figure 3.2.3.2). The photographs taken on Day 28 show perhaps the greatest difference in decomposition state between the indoor piglet (Figure 3.2.3.1) and the outdoor piglet (Figure 3.2.3.2). In run 5, oviposition was delayed by three days in the indoor piglet, although thereafter larval development (Tables 3.3.2.20 & 3.3.2.21) and decomposition (Figure 3.2.3.16) progressed at a similar rate.

During this run the temperatures indoors and outdoors were fairly similar, with a mean of 18.9°C outdoors and 22.7°C indoors. In run 4, oviposition outdoors lagged behind indoor oviposition by nine days, due to the temperature outdoors fluctuating around 5°C for the first two weeks, resulting in a significant delay in decomposition of the outdoor piglet, in comparison to the indoor piglet (Figure 3.2.3.12). Similarly, in run 2, although egg-laying was delayed by 12 days in the indoor piglet, the outdoor ambient temperatures were around 6°C for the first two weeks of the experiment, resulting in very slow development of the larvae. By Day 47, however, the piglets were both decomposed (Figure 3.2.3.5) and the blowflies on both piglets were still in the pupal stage (Tables 3.2.3.8 & 3.2.3.9).

In all five runs, the internal body dataloggers in the outdoor piglets followed the outdoor ambient datalogger temperatures very closely. The internal body dataloggers in the indoor piglets, however, showed increased temperatures above ambient due to the internal larval masses, the highest temperature reached being 43°C in the rectum of Piglet 5i (Figure 3.2.3.17).

3.3 Blowfly larval development in a rural environment

3.3.1 Aims

The aim of this study was to decompose a number of piglet cadavers in a rural location near London, to identify the most common blowfly species, and to compare the estimated minPMIs with published data. Decomposition of the piglets was also recorded, as well as larval mass temperatures, as a comparison with the piglets decomposed in an urban environment. The hypothesis was that there would be more decomposition of the piglets in a rural environment due to the greater species diversity, with respect to insect succession.

3.3.2 Materials and methods

3.3.2.1 Study site

The chosen site was the Royal Botanic Gardens, Kew, covering approximately 300 acres (120 hectares), situated to the West of London in Surrey, and designated a UNESCO World Heritage Site in July 2003. The area within Kew Gardens used for this study was the Conservation Area, situated at the North West corner of the Gardens, with a golf course to the South and the River Thames to its West (Figure 3.3.2.1). The Conservation Area is managed as a nature reserve, with native plants and animals encouraged through the employment of traditional methods and management techniques such as coppicing. The area consisted of a small pond, small areas of open grassland, brambles and some mature trees.

Figure 3.3.2.1 Map of Royal Botanic Gardens, Kew (www.kew.org/) where experiments were carried out. The experimental site was the conservation area (outlined in red).

3.3.2.2 Animal model

Neonate stillborn piglets were purchased from a pig farmer in Oxfordshire. Two piglets were put out in May 2004, and four in August 2005. In each case, each piglet was enclosed in a collapsible metal dog cage to guard against vertebrate scavengers such as badgers, foxes and crows. The cages measured 61cm height x 61cm width x 122cm length, with a hinged door at one end. Metal tent pegs were used to secure each cage to the ground.

In the first experiment, one piglet weighing 840g (Piglet 1A) was placed in a plastic bowl within the cage, and the cage covered over with plastic, to prevent the bowl from filling up with rainwater. The second piglet weighing 780g (Piglet 1B) was placed directly onto the gridded base of the cage, and therefore in direct contact with the ground. Although treated differently, this was the same protocol used in the experiments at the Natural History Museum for indoor vs. outdoor cadavers (outlined in Section 3.2). Sticky fly paper was attached to the inside of each cage to trap insects visiting the carcasses, and was also attached to the inside of a third cage which did not contain a cadaver, used as a control. The cages were spaced ten metres apart in a triangular formation (Figure 3.3.2.2), ensuring that they were far enough apart whilst still being within the same environmental location.

Figure 3.3.2.2 Layout of cages in Experiment 1: piglet 1A (in plastic bowl and cage covered with black plastic) & piglet 1B (placed directly into a cage)

In the second experiment, two of the four piglets were placed in plastic bowls within the cages, and the cages covered with plastic (Piglets 2A & 2C), and two were placed directly on the base of the cage (Piglets 2B & 2D). The four cages were placed in a row, 10 metres apart (Figure 3.3.2.3). The piglets weighed 1600g (2A), 1900g (2B), 1200g (2C) and 1900g (2D).

Figure 3.3.2.3 Layout of cages in Experiment 2: piglets 2A & 2C (in plastic bowls and cages covered with black plastic) & piglets 2B & 2D (placed directly into cages)

3.3.2.3 Sampling procedure

For the duration of each experiment, a Tinytag® datalogger was placed at the site, to record hourly temperatures. Each piglet also had a probe datalogger inserted in the mouth and rectum during the duration of the experiment to record internal body temperatures. The temperature of any visible larval masses were recorded at each visit, using a non-invasive infra-red thermometer.

During the period of Diptera colonisation, sampling was carried out from Day 1-9, Day 11-12 and on Day 18 (Experiment 1) and from Day1-10 (Experiment 2), where Day 1 was the day the piglets were laid out. On each occasion, notes were taken of the state of decomposition of each cadaver, photographs were taken, and samples of adult and immature insects were collected using forceps. All adult samples collected were either preserved in 80% ethanol or kept as dry samples, and identified using a binocular microscope at magnification up to x25 and identification keys by Smith (1986). Diptera larvae were collected from larval masses on each cadaver and within an hour after being taken back to the NHM laboratory, killed by immersion in freshly boiled water and then placed in 80% ethanol. Ten larvae from each sample were selected and then identified and measured under a binocular microscope up to x25 magnification, using an eyepiece graticule. The graticule units were then converted to millimetres, and the mean length of each sample was calculated.

3.3.3 Results

These are outlined below for Experiment 1 in section 3.3.3.1 (Piglets 1A & 1B) and Experiment 2 in section 3.3.3.2 (Piglets 2A, 2B, 2C & 2D).

They include daily observations, photographs of each cadaver taken at regular intervals, internal probe and ambient datalogger temperatures, infrared temperatures, and larval development.

3.3.3.1 Experiment 1

Table 3.3.3.1 Field observations recorded at each visit during Experiment 1

Day	Date & time	Piglet 1A (bowl, covered)	Piglet 1B (ground, uncovered)
1	22nd May 2004 17:00 [started dataloggers]	Pig put out; *Calliphora* and *Lucilia* visiting within minutes	Pig put out; *Calliphora* and *Lucilia* visiting within minutes
2	23rd May 2004 16:00	Lots of *Calliphora* and *Lucilia* ovipositing under body, in mouth, under front legs; egg masses under back legs and in ears	Large Sarcophagid, lots of *Lucilia*; egg masses in mouth, between back legs, on stomach, in folds of neck, on chest and right ear
3	24th May 2004 14:30	Bloating; no eggs hatched; *Calliphora* and *Lucilia* feeding in mouth and on body	*Lucilia* feeding from nose; *Calliphora* ovipositing under body; big new egg mass under chin; no larvae hatched yet; Sarcophagid feeding in nose and chasing off other flies
4	25th May 2004 14:00	Eggs on stomach hatched in larvae; *Calliphora* and *Lucilia* present	Sarcophagid in mouth, and *Lucilia*; some egg masses hatched on stomach, chest and in nostrils.
5	26th May 2004 14:00	Lots of *Lucilia* and *Calliphora*; larval mass in mouth, under body, behind ear and on stomach; new egg masses on neck and under front leg	Large 2nd instar larval mass in mouth and nose; lots of *Lucilia* and Sarcophagids; 1st instars in rectum and on stomach; 1st/2nd instars on chest
6	27th May 2004 15:30	Very bloody, 100s of drowned eggs; lots of fly activity and many more on fly paper; mouth empty except for adults	Large 2nd instar larval mass in mouth and neck; *Calliphora* and *Lucilia* feeding in mouth and rectum; lots of small flies
7	28th May 2004 13:30	Lots of *Lucilia* and *Calliphora*; also parasitic Hymenoptera; still very bloody, guts visible with lots of flies feeding on them	Large larval mass behind ear, in chest and stomach; more beetle larvae; no Sarcophagids; a few *Lucilia* and *Calliphora*
8	29th May 2004 15:00	Still lots of flies; larval mass in abdomen; lots of fluid in bowel, with *Calliphora* and *Lucilia* adults still feeding; also small flies, ants and earwigs	Skin and bones visible; larvae not yet post-feeding but still actively feeding; lots of beetles, earwigs and flying ants
9	30th May 2004 17:00	Larvae feeding in fluid in bowel; beetles feeding on wandering larvae; a few post-feeding larvae under bowel	10 out of 12 pitfall traps removed (by animals?) but post-feeding larvae still in holes in the earth
11	1st June 2004 13:30 [removed dataloggers]	Body disintegrated; 100's of larvae in watery fluid in tray under bowel	A few post-feeding larvae and pupae in pitfall holes; small flies still on body
12	2nd June 2004 13:00	Earwigs, black beetles and red ants on tray with post-feeding larvae; wandering larvae found up to 1.5m away from cage (n=111)	Large black beetles, tiny black flies, a few earwigs; a few more wandering larvae in pitfalls
18	8th June 2004 13:00	No more black beetles or earwigs; just black/brown beetle larvae; no flies apart from 1 Sarcophagid, and a wasp	Body dried out on top; soil and grass underneath blackened; black/brown beetle larvae, a few small black flies, and a wasp

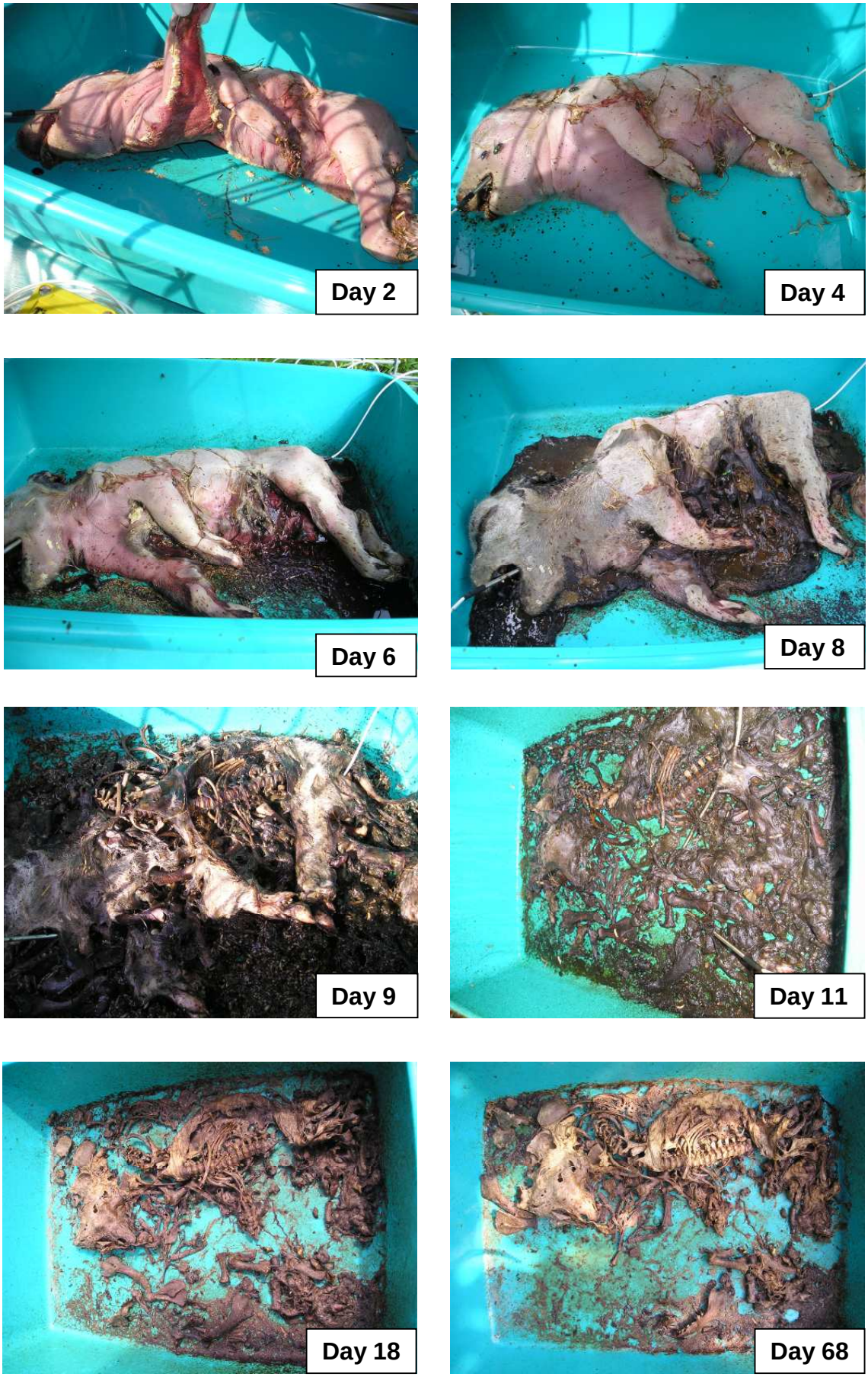


Figure 3.3.3.1 Photographs taken during decomposition of Piglet 1A, stating day of experiment

Figure 3.3.3.2 Photographs taken during decomposition of Piglet 1B, stating day of experiment

Table 3.3.3.2 Experiment 1 datalogger statistics (Day 1 14:00 to Day 11 12:00)

Datalogger	Min (°C)	Max (°C)	Range (°C)	Mean (°C)	SD
Ambient	0.8	27.6	26.8	14.51	±6.62
Piglet 1A (mouth)	4.1	30.2	26.1	16.55	±5.92
Piglet 1A (rectum)	3.9	29.8	25.9	16.13	±6.11
Piglet 1B (mouth)	2.3	40.4	38.1	18.75	±8.56
Piglet 1B (rectum)	4.6	32.2	27.6	17.76	±7.15

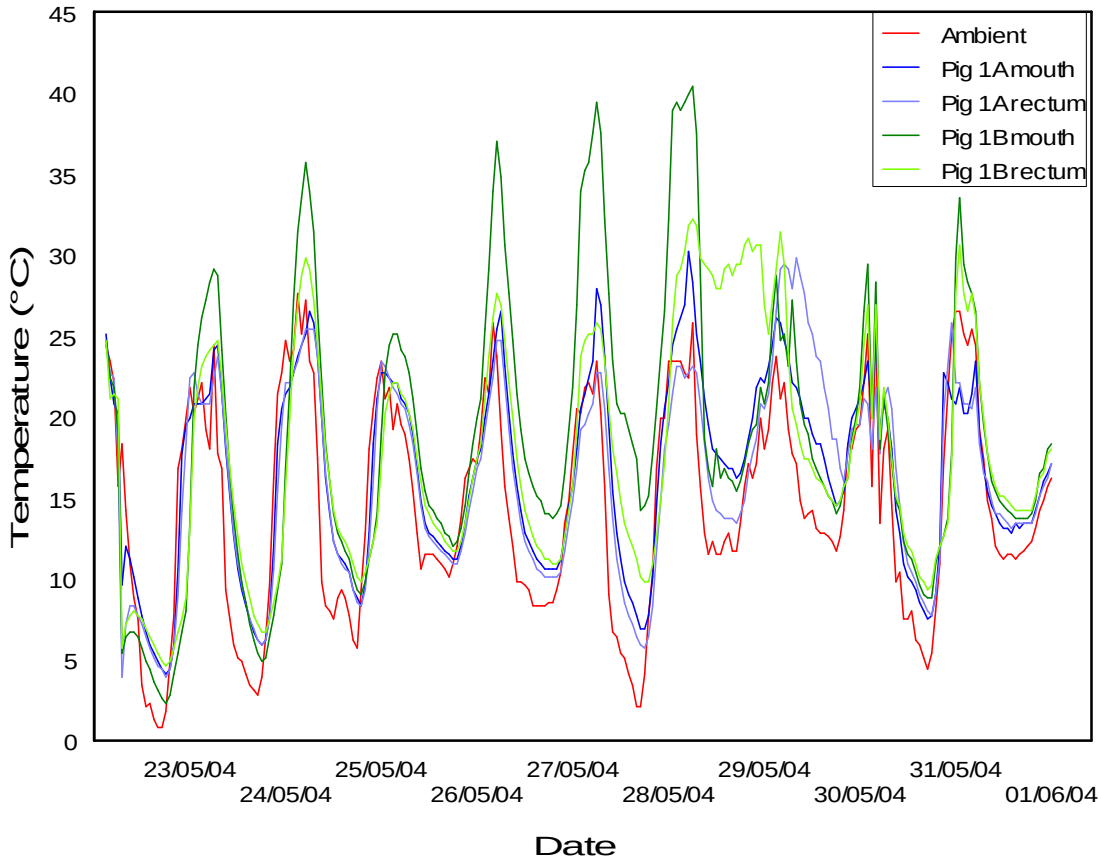


Figure 3.3.3.3 Experiment 1: datalogger temperatures for Pig 1A and Pig 1B

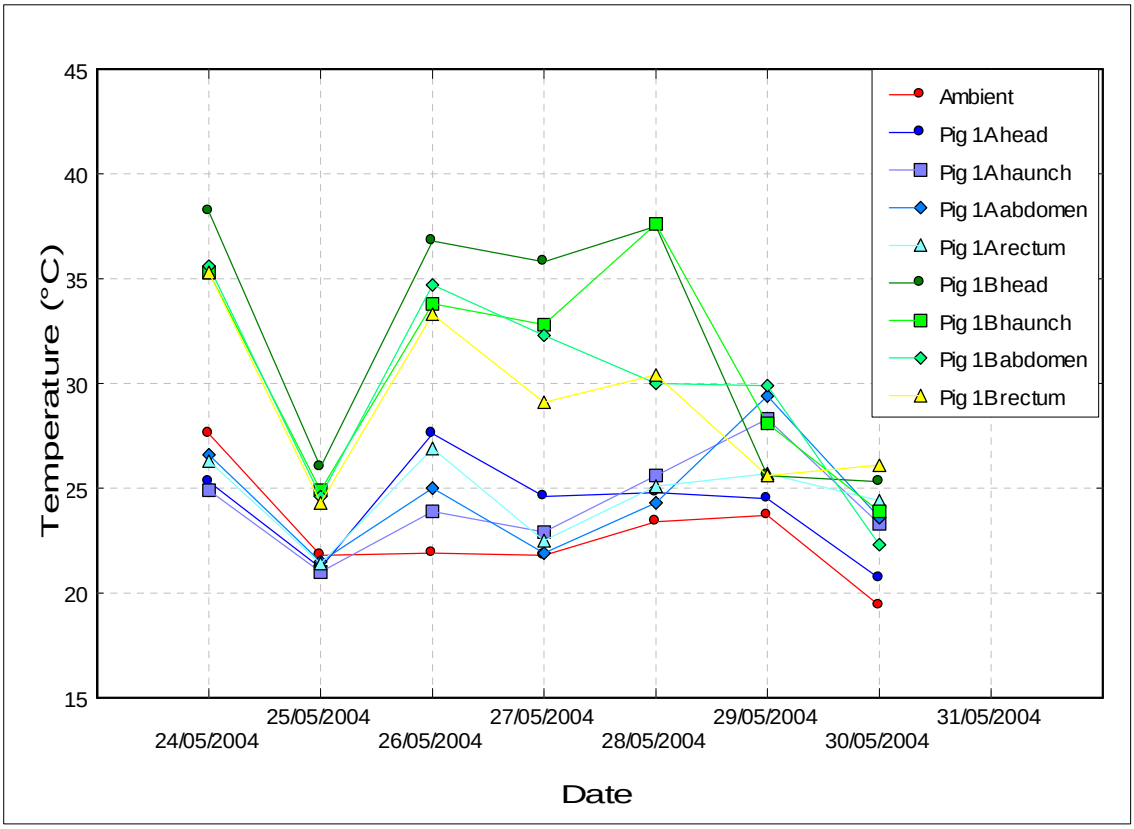


Figure 3.3.3.4 Experiment 1 infrared temperatures recorded on larval masses on two piglets

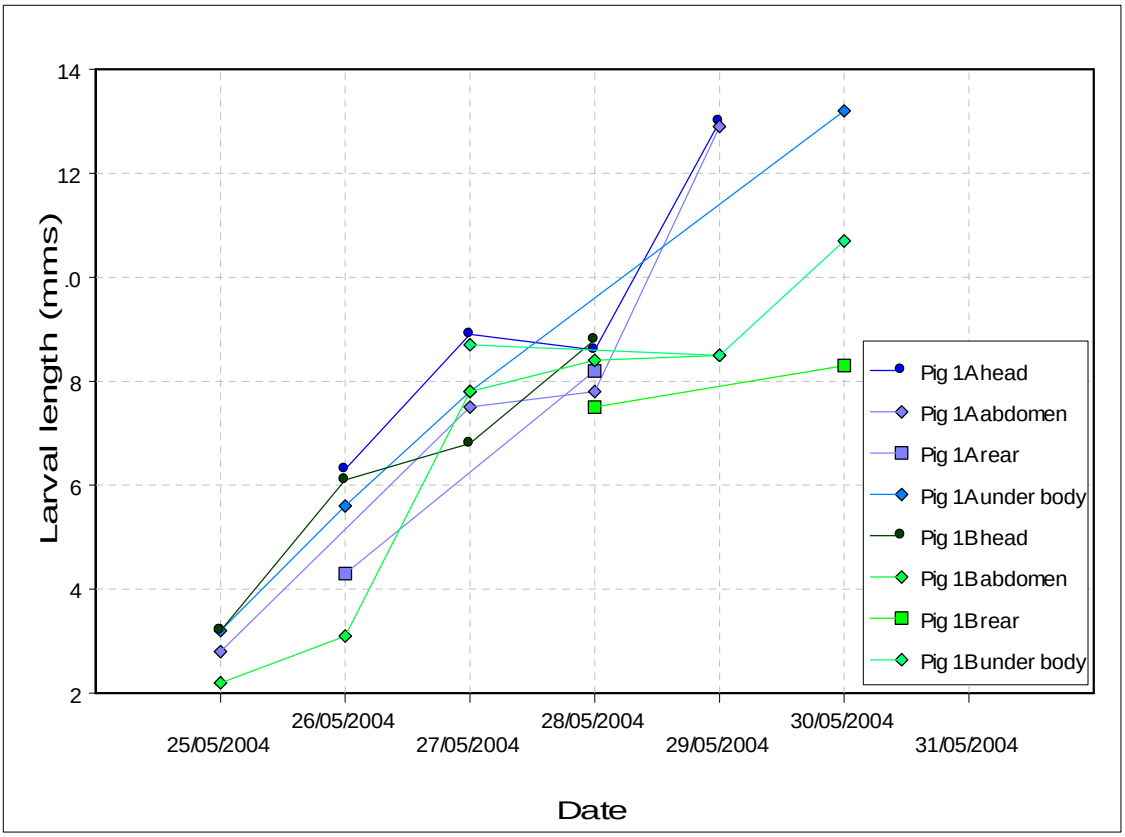


Figure 3.3.3.5 Experiment 1 larval lengths of *Lucilia sericata* collected off two piglets

3.3.3.2 Experiment 2

Table 3.3.3.3 Field observations recorded at each visit during Experiment 2

Day	Date & time	Piglet 2A (bowl)	Piglet 2B (ground)	Piglet 2C (bowl)	Piglet 2D (ground)
1	5th Aug 2005 13:00 [started dataloggers]	No flies	*Lucilia*	2 *Calliphora*	No flies
2	6th Aug 2005 16:00	Lots of *Lucilia*, esp. on head and in mouth; lots of eggs between legs and under body	Lots of *Lucilia* and eggs, esp. under body	Lots of *Lucilia* and eggs, esp. under body; bloated	No bloat; a few *Lucilia*; eggs in mouth and mainly between legs
3	7th Aug 2005 18:30	Some 1st instars in mouth and between legs; *Lucilia* still on body; little bloat	*Lucilia* and Muscids; bloat; 1st instars in mouth; a few newly hatched under body	Lots of *Lucilia*; lots of eggs on haunches; 1st instars in mouth and between legs	Huge numbers of eggs under chin; 1st instars in mouth; some hatched under body
4	8th Aug 2005 16:00	Slightly bloated; lots of *Lucilia*	Lots of *Lucilia* & *Calliphora*; large larval mass in mouth; beetles and earwigs under body	Lots of *Lucilia*	Lots of *Lucilia* and *Calliphora*
5	9th Aug 2005 15:00	2nd instar larval mass in mouth; still 1st instars between legs; hister beetles	Full bloat; lots of 1st and 2nd instar larvae in mouth and neck; small larval mass on stomach; beetles under body	2nd instar larval mass on abdomen	2nd instar larval mass in mouth; egg masses on chest
6	10th Aug 2005 15:30	Large larval mass on face, and along body/bowl interface	In full bloat; larval masses inside body cavity and mouth; layer of larvae seen under epidermis	Large larval mass in thorax and neck; lots of flies	Large larval mass in abdomen; various beetles present
7	11th Aug 2005 17:30	Large larval masses in abdomen and neck, but larvae still small	Body flattened, soft flesh mainly consumed; larvae underneath body	Larvae post-feeding and grouped in corner of bowl	Top half of body completely skeletal; large mass of larvae at rear end
8	12th Aug 2005 16:00	Larval mass on neck; a few larvae under bowl; *Lucilia* adults still present	Just a carcass remaining; a few larvae moving off body	Post-feeding larvae in corner of cage, many still in bowl and under body	Very little remaining of piglet; post-feeding larvae leaving in all directions
9	13th Aug 2005 15:00	Large larval mass still on body and a few under bowl, moving off body	No larvae visible on body, most underneath have gone; a few post-feeding larvae on ground	Larvae still in and under bowl, and in metal tray; no larvae visible on ground; a number of dead larvae in bowl	A couple of small groups of larvae on ground where piglet was laying; most post-feeding larvae left the previous day

Day	Date & time	Piglet 2A (bowl)	Piglet 2B (ground)	Piglet 2C (bowl)	Piglet 2D (ground)
10	14th Aug 2005 15:00	Lots of larvae have left the body, although many are still on it; larvae on ground moving away and under bowl	A few small larval masses on body; most larvae have left; lots of beetles under body	A lot of dead larvae; a few alive under bowl; no pupae visible	A few tiny underdeveloped larvae can be seen; beetles and small black flies present
11	15th Aug 2005 15:00	Lots of small flies on body; larvae under body	Body mummified; lots of beetles under body; few larvae as most have left	Larvae all dead; no pupae visible; some post-feeding larvae under body	Post-feeding and pre-pupae found in soil surrounding body
12	16th Aug 2005	Still pre-pupae, no pupae	[no comment]	Larvae collected from under bowl	[no comment]
13	17th Aug 2005 15:30	Non-Calliphorid larvae collected from under body and under bowl	[no comment]	[no comment]	Pre-pupae & pupae collected: some put in cage, some taken back to lab
14	18th Aug 2005 14:30 [removed dataloggers]	Non-Calliphorid larvae collected from under body and under bowl	[no comment]	[no comment]	[no comment]

Figure 3.3.3.6 Photographs taken during decomposition of Piglets 2A, 2B, 2C & 2D, stating day of experiment

Table 3.3.3.4 Experiment 2 datalogger statistics (Day 1 11:00 to Day 14 14:00)

Datalogger	Min (°C)	Max (°C)	Range(°C)	Mean (°C)	SD
Ambient	8.0	30.6	22.6	16.99	±4.91
Piglet 2A (rectum)	9.3	35.7	26.4	18.25	±4.94
Piglet 2B (mouth)	10.4	41.5	31.1	19.66	±6.41
Piglet 2B (rectum)	9.0	39.4	30.4	18.76	±6.00
Piglet 2C (mouth)	9.8	31.8	22.0	17.70	±4.88
Piglet 2C (rectum)	9.0	35.7	26.7	17.39	±5.01
Piglet 2D (mouth)	10.1	35.2	25.1	20.33	±4.75
Piglet 2D (rectum)	10.4	35.2	24.8	19.89	±4.70

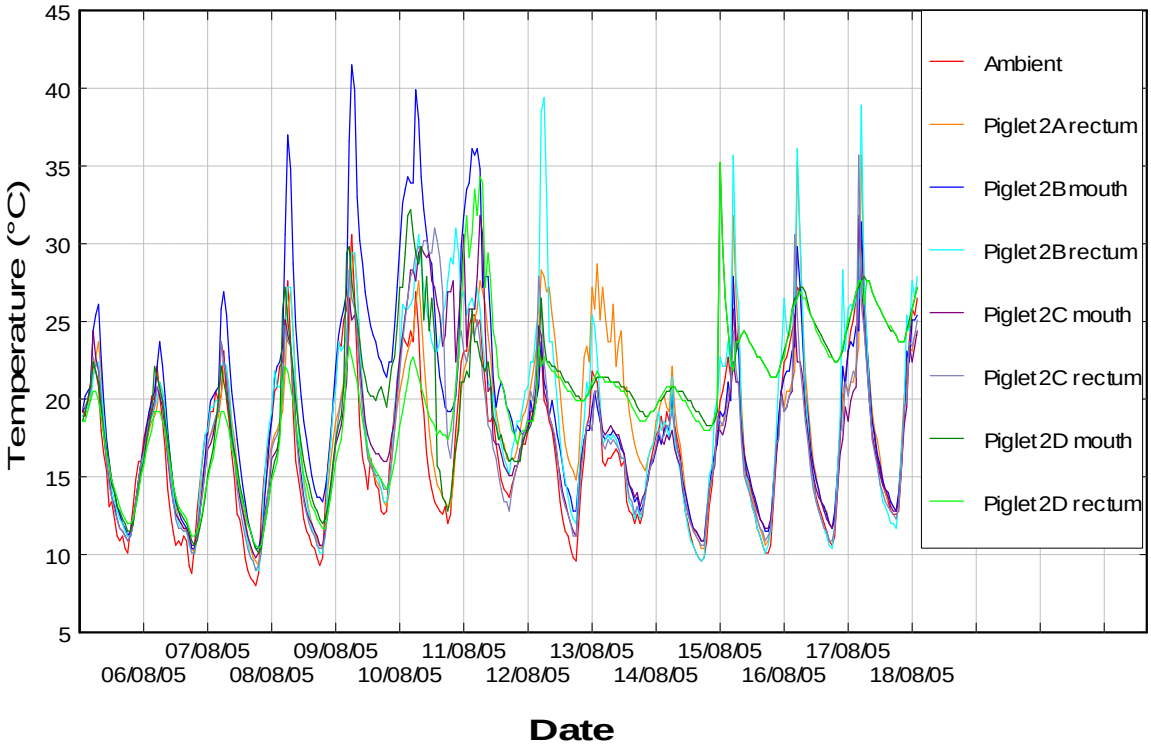


Figure 3.3.3.7 Experiment 2 datalogger temperatures recorded from four piglet cadavers

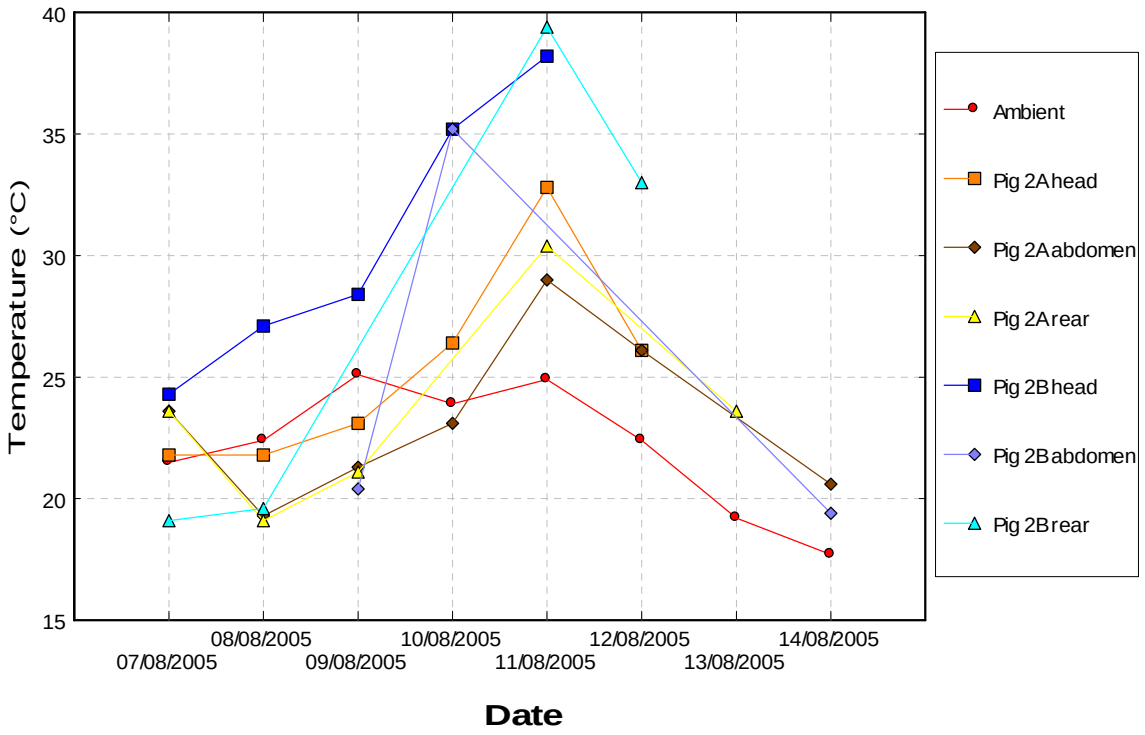


Figure 3.3.3.8 Experiment 2 infrared temperatures recorded for Piglets 2A & 2B

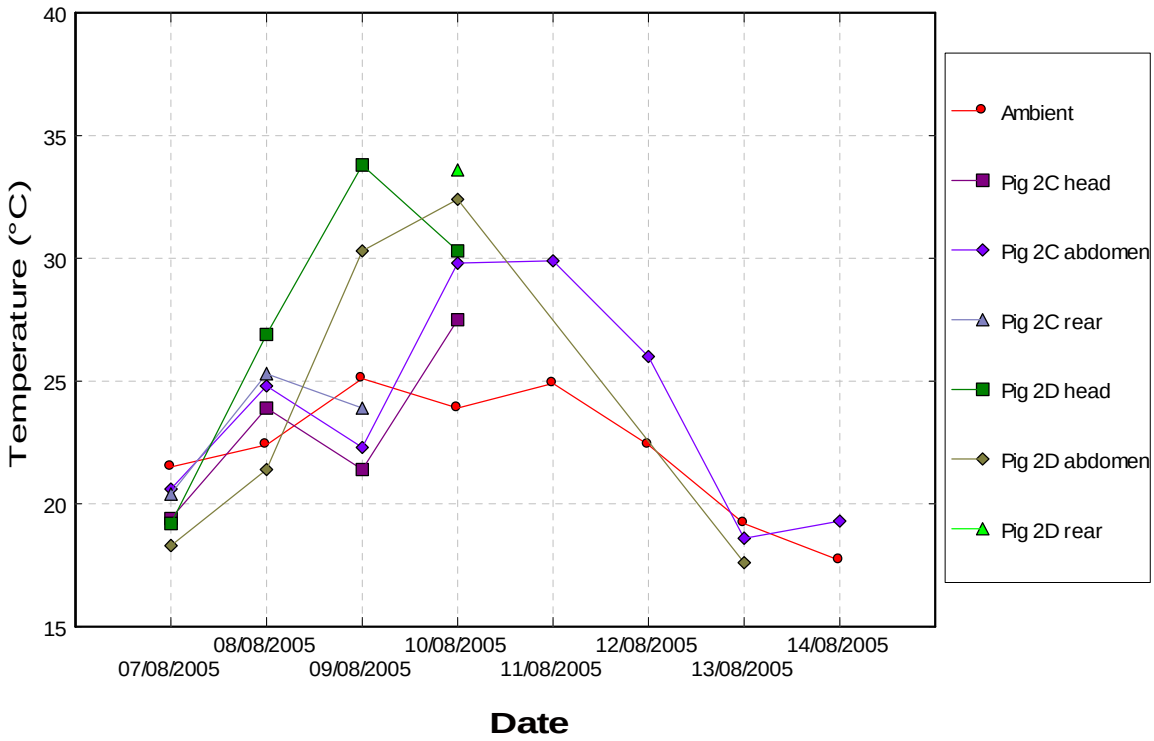


Figure 3.3.3.9 Experiment 2 infrared temperatures recorded for Piglets 2C &2D

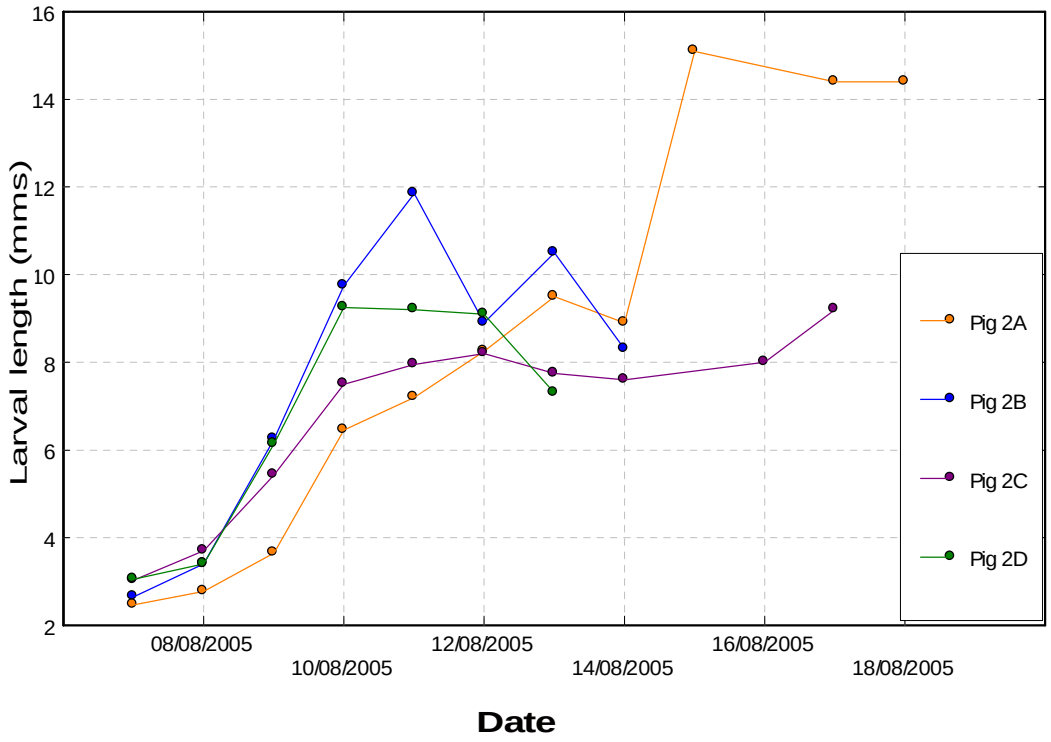


Figure 3.3.3.10 Experiment 2 mean larval lengths of *Lucilia sericata* for Piglets 2A, 2B, 2C & 2D (n=10)

The predominant species of blowfly in both Experiments 1 and 2 and all cadavers was *Lucilia sericata*, with *Calliphora vicina* and Sarcophagids also present. In Experiment 1, both piglets attracted blowflies within minutes of being put out, and by Day 2 there were already a large number of egg masses on the cadavers. In Experiment 2, only two of the four piglets attracted blowflies when they were first put out, but by Day 2 all four cadavers had egg masses on them. In Experiment 1, Piglet 1A (Figure 3.3.3.1) appeared to have lost more biomass, specifically skin and fur, by Day 11, than had Piglet 1B (Figure 3.3.3.2), and the larval mass temperatures in Piglet 1A were about 10°C higher than in Piglet 1B. In Experiment 2, Piglet 2D decomposed faster than the other 3, which still had some skin attached to the bones after 28 days, whereas Piglet 2D was skeletal by Day 10. The mean ambient temperature was slightly higher in Experiment 2 (16.99°C) than in Experiment 1 (14.51°C). In Experiment 1, larval mass temperatures, measured using an infrared thermometer, reached 15°C higher in Piglet 1A than the ambient temperature, and in Experiment 2 those of Piglet 2B were 11°C higher than the ambient.

3.4 Longevity of Calliphorid puparia in a rural environment

3.4.1 Aims

The aim of this study was to determine how long empty puparia of a common UK species of blowfly (*Calliphora vicina*) will survive in a natural outdoor environment in the UK before they disintegrate and can no longer be located and recovered. In setting up this study, a number of factors were considered:

a) Chronological differences – will the puparia disintegrate more, the longer the time period?
b) Meteorological effects – are there particular weather conditions or seasons which help or hinder the location and recovery of puparia?
c) Placement of puparia – do puparia stay intact longer depending on location i.e. on the surface, buried, or in the leaf/soil interface?
d) Position along transect – are puparia more likely to disintegrate along a particular area of the transect?

3.4.2 Materials and methods

3.4.2.1 Study site

The site chosen was a wooded area in a private garden in Hampshire, southern UK. The flora consists of snowdrops, daffodils, bluebells and ground elder in succession, illustrated in Figure 3.4.2.1 below. There are a number of large trees, including beech, holly and conifers. The land is not cultivated and therefore has a high nutrient content due to the annual fall of leaf litter. The area is known to be used sporadically, but not heavily, by people picking flowers, visiting the nearby Pet Cemetery, and by the three resident dogs. The area is similar to that where bodies have been discovered in the UK, i.e. secluded woodland, rarely used except perhaps by bird watchers and dog walkers.

Figure 3.4.2.1 The experimental area in Hampshire, photographed in May (left) and October (right)

3.4.2.2 Experiment set-up

In May 2004, a non-stretchable nylon string was strung between three trees in an approximate straight line (Figure 3.4.2.1), and marked out every metre over a 50 metre length. Each metre was taped with a numbered label, wrapped in sticky tape to waterproof it, and attached to the transect. The point where the string was wrapped around the trees was marked both on the string itself and on the tree with a black marker pen (Figure 3.4.2.2). A loose string one metre in length was tied around the main transect, enabling it to be moved from one marker point to another, in order to measure a distance of one metre on each side of the transect.

Figure 3.4.2.2 Transect around tree (left) and numbered labels (centre and right)

At each metre mark, three sets of 40 empty puparia were placed: one directly under the transect, and one on each side, exactly a metre away from the transect. The puparia were those of the bluebottle blowfly *Calliphora vicina* (Diptera: Calliphoridae) which had been reared from wild flies on pigs laid out at

the Natural History Museum, and from which flies had emerged no more than 15 weeks previously. The puparia were counted out in batches of 40 x 3 x 50 into plastic containers, giving a total of 6000 puparia. At each metre mark, the three batches were laid out in one of three ways, shown in Figure 3.4.2.3: a) on the surface, b) buried to a depth of approximately 5cm, or c) placed between the leaf litter and the soil. The placement of these was varied at each metre mark, with a total of 6 variations being possible:

1) Buried – Surface – Leaf/soil
2) Buried – Leaf/soil – Surface
3) Surface – Leaf/soil – Buried
4) Surface – Buried – Leaf/soil
5) Leaf/soil – Surface – Buried
6) Leaf/soil – Buried – Surface

Once the puparia were all laid out, the transect was removed to ensure that the area was utilised normally and did not receive either undue attention, or be avoided altogether.

Figure 3.4.2.3 Placement of puparia
Indicated by white arrows

A) surface
B) leaf/soil
C) buried

3.4.2.3 Sampling procedure

At intervals of approximately three to six weeks over the next six years, the puparia were collected at each metre mark, randomly chosen, for each of the three batches (surface, leaf/soil, buried). On each occasion, the transect was set up as before, and the puparia searched for. Each site was searched for approximately 20 minutes, and if no more puparia were found after about 5 minutes, the search was ended. The assumption was that once one puparium was located, any still remaining would be easily found if they had not disintegrated. Searching was carried out with a combination of hand-searching with forceps, sieving small amounts of leaf litter and soil, or placing small amounts of leaf litter and soil on a white sheet of paper and searching by hand. Usually the site directly under the transect was searched first to ensure that the correct area was located before searching the areas one metre either side of the transect. Any recovered puparia were placed in individual re-sealable plastic bags and labelled with their location. The number of puparia collected at each site was counted, with an estimate given for fragments. All puparia were recovered and counted by the same person, so there was consistency throughout the sampling method and in estimation of remaining puparia.

3.4.3 Results

The raw data are given in Appendix I, Table 3.4.3.1, showing date of collection, number and percentage of puparia out of 40 recovered from each sample site, plus mean percentage for each transect marker.

3.4.3.1 Chronological differences

The mean percentage of puparia recovered at each transect marker over the six year period (Figure 3.4.3.1) indicates a gradual decrease over time. Within the first 18 months of sampling, the mean percentage of puparia recovered at each transect is between 80%-90% on four sampling occasions, but decreases to a maximum of just over 60% after this time, and is as low as 0% for much of the last four years. The equation of the polynomial trend line is R^2=0.6642,

indicating a relatively weak fit of the regression lines to the points i.e. there is a positive, though not particularly strong relationship between the total percentage of puparia and the date on which they were recovered.

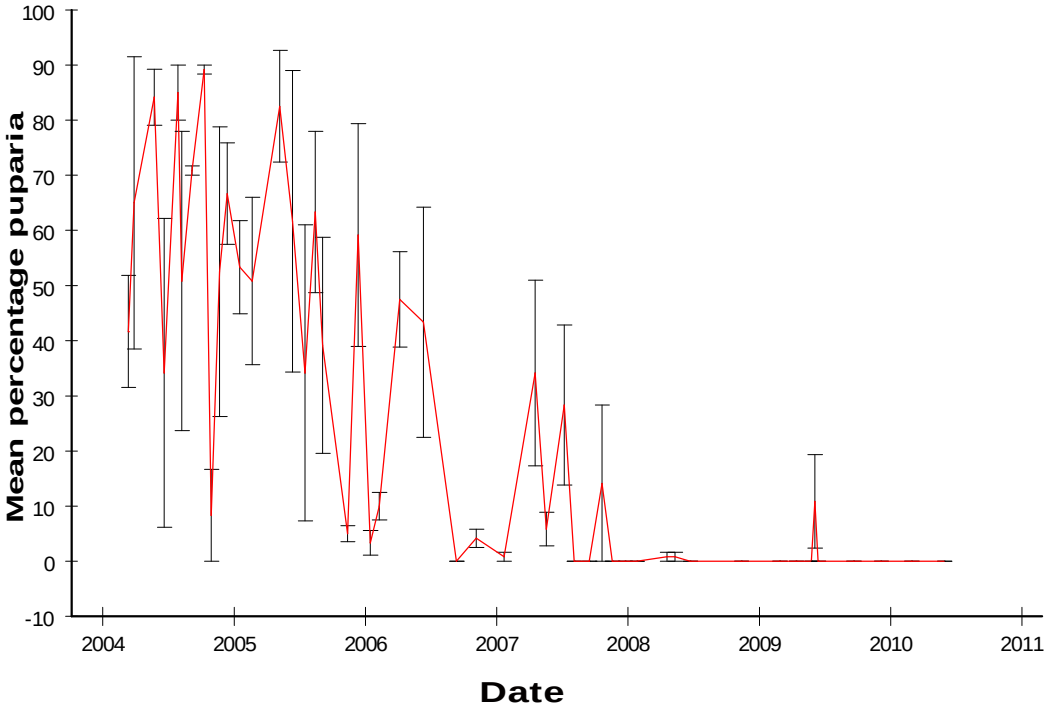


Figure 3.4.3.1 Mean percentage of puparia recovered at each metre transect from 2004 to 2010

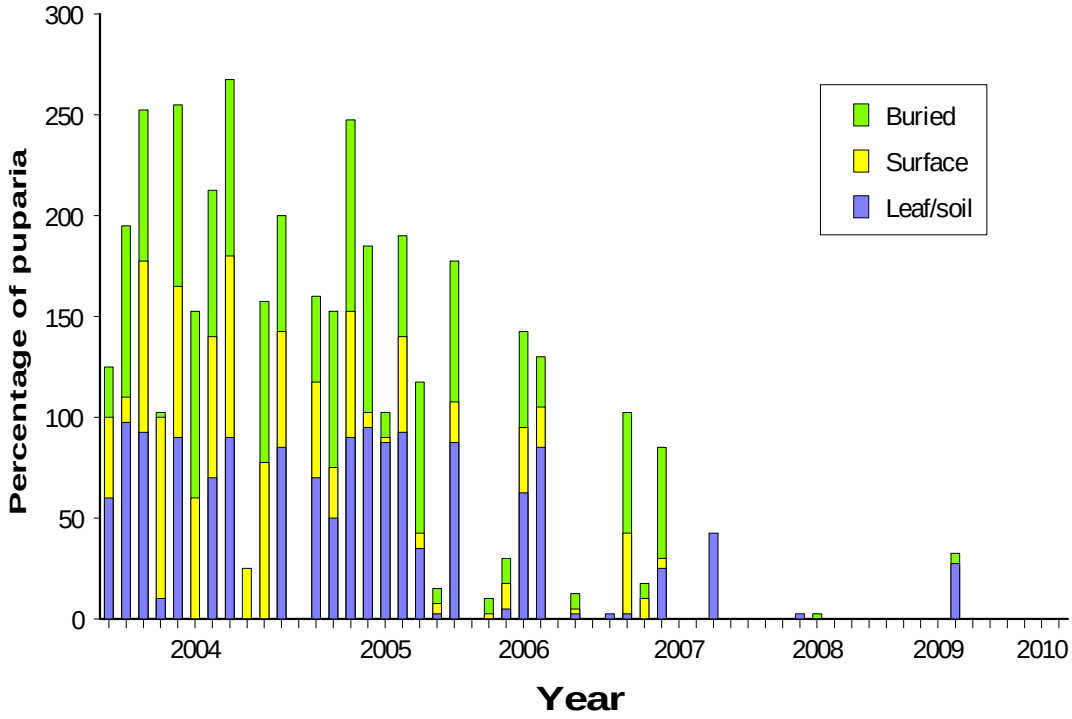


Figure 3.4.3.2 Samples over time: buried (green), surface (orange), leaf/soil (blue) from a 50m transect

In addition, the puparia visibly disintegrated over the six year period. Those collected in the first year were intact (Figure 3.4.3.3), whereas those collected in later e.g. the fifth year, were very fragmented (Figure 3.4.3.4).

Figure 3.4.3.3 Intact puparia recovered in first year of experiment

Figure 3.4.3.4 Fragmented puparia from fifth year of experiment

3.4.3.2 Meteorological effects

In order to assess the effect of weather conditions, the collecting period was divided up into four seasons: spring (March to May, n=13), summer (June to August, n=12), autumn (September to November, n=13) and winter (December to February, n=12). The greatest proportion of puparia was recovered during the summer season, with the least during the winter (Figure 3.4.3.5). Apart from during the autumn period, the greatest proportion of recovered puparia was those that had been buried, with the surface puparia being the least number recovered. In autumn, however, the least number of buried puparia were recovered.

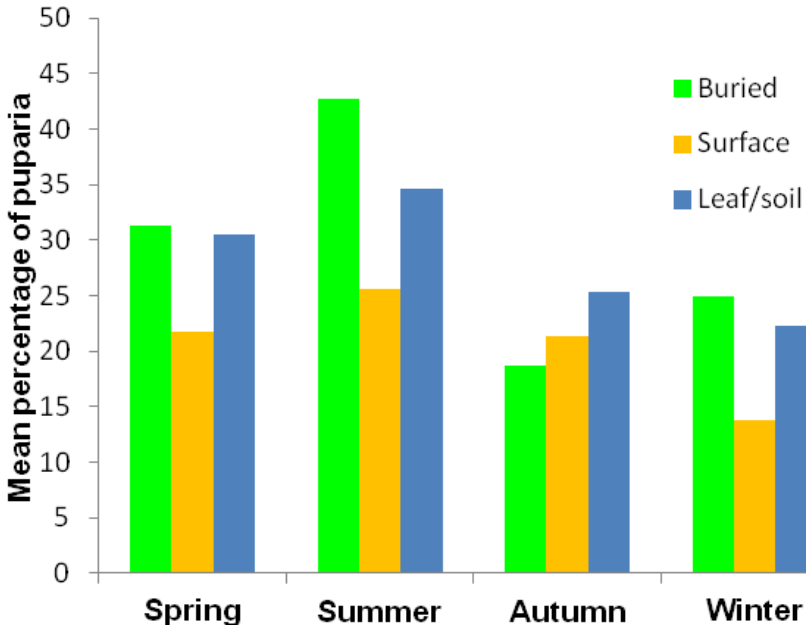


Figure 3.4.3.5 Samples of puparia collected in four seasons: spring (March to May, n=13), summer (June to August, n=12), autumn (September to November, n=13), winter (December to February, n=12)

A chi-square test indicates that there is no significant difference between the Autumn and Winter results (2.0172, p=0.1555), but that there is a significant difference between all the other results: Spring and Summer (28.5383, p<0.00001), Spring and Autumn (33.9417, p<0.00001), Spring and Winter (54.6841, p<0.00001), Summer and Autumn (132.2315, p<0.0001), Summer and Winter (173.1106, p<0.0001).

3.4.3.3 Placement of puparia

The least number of puparia, just over 20%, were recovered from the surface, with similar numbers, just under 30%, recovered from those which were buried or placed between the leaf/soil interface (Figure 3.4.3.6).

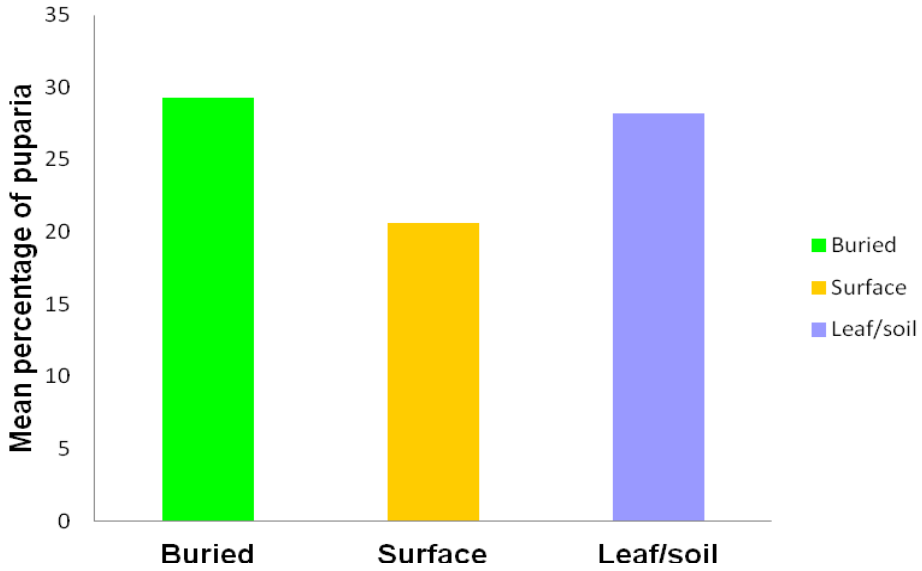


Figure 3.4.3.6 Samples collected from three placement: buried (green), surface (yellow) and leaf/soil (blue)

A chi-square test indicates that there is no significant difference between the buried and leaf/soil results (1.089, p=0.2967), but that there is a significant difference between the buried and surface results (90.2734, p<0.00001) and between the surface and leaf/soil results (56.3054, p<0.0001).

3.4.3.4 Position along transect

The total number of puparia recovered throughout the six year period with all samples combined (buried, surface, leaf/soil) are shown in Figure 3.4.3.7. There does not appear to be any particular bias along the length of the transect.

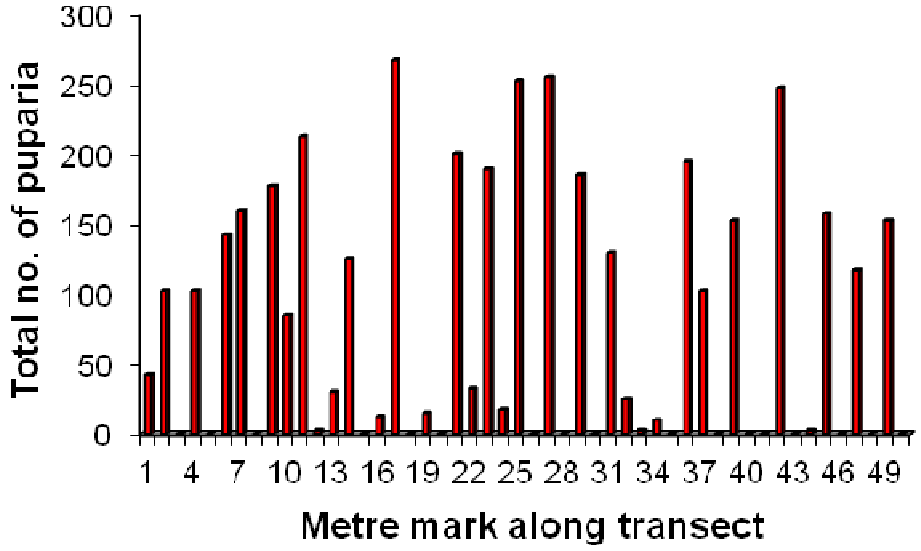


Figure 3.4.3.7 All puparia samples collected along transect, from 1-50 metres

Similarly, there does not appear to be any bias in each of the three samples, as shown in Figures 3.4.3.8 (buried), 3.4.3.9 (surface) and 3.4.3.10 (leaf/soil).

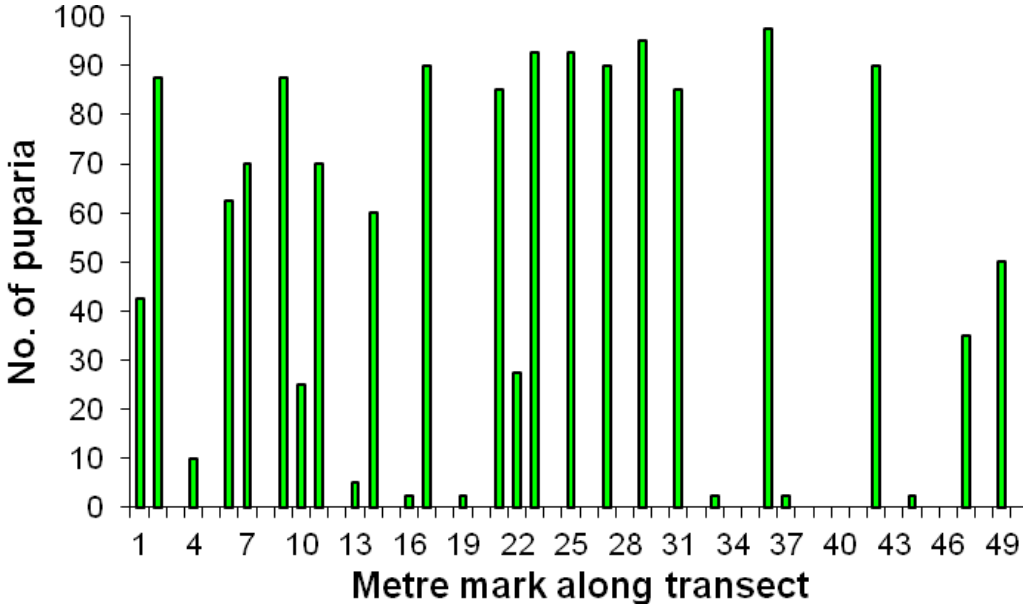


Figure 3.4.3.8 Buried puparia samples collected along transect, from 1-50 metres

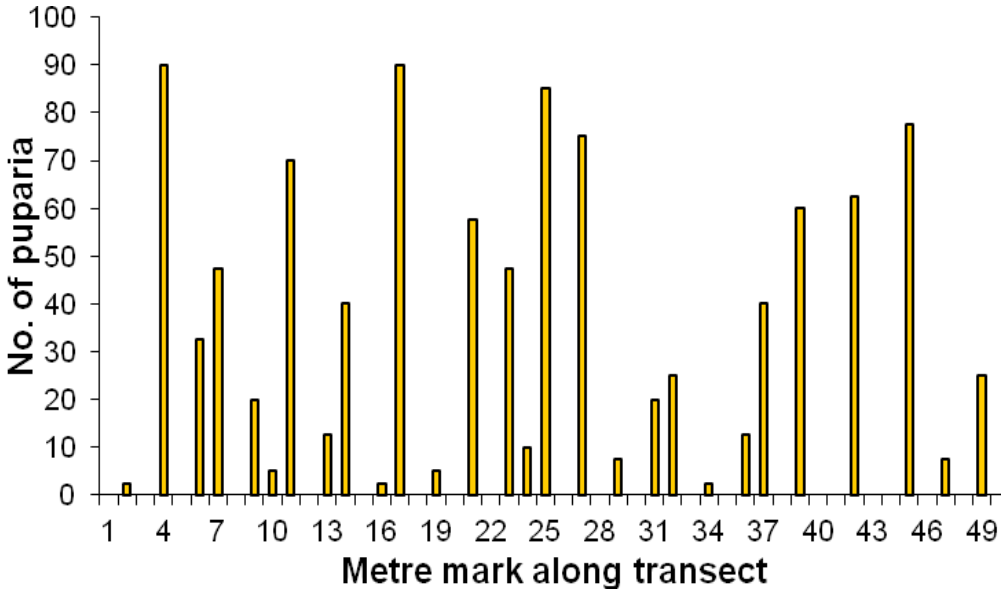


Figure 3.4.3.9 Surface puparia samples collected along transect, from 1-50 metres

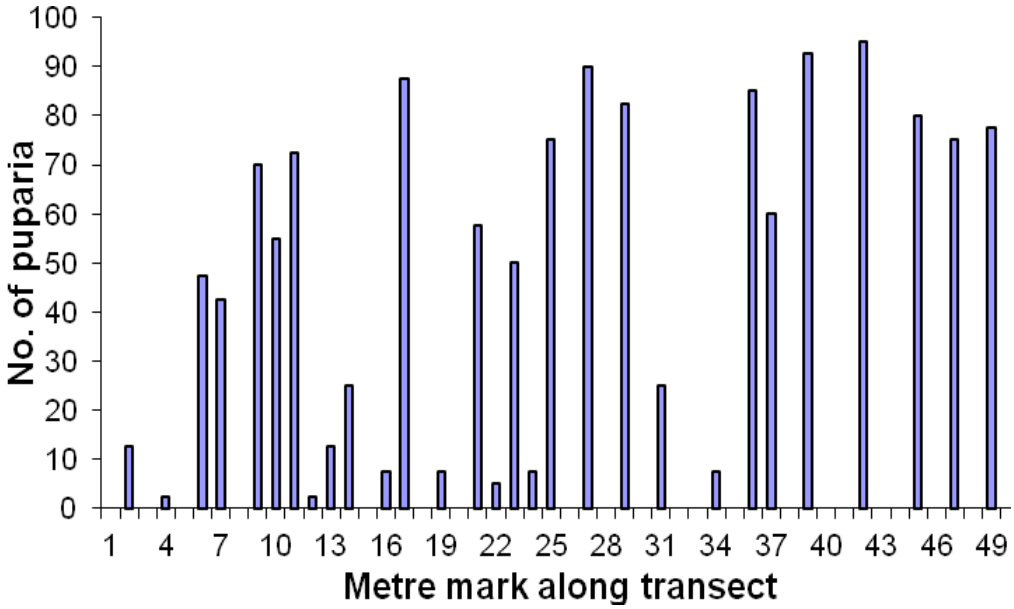


Figure 3.4.3.10 Leaf/soil puparia samples collected along transect, from 1-50 metres

During the first two years, a significant proportion of puparia were recovered, 70-90% at most sampling sites, and the recovered puparia were largely intact, as in Figure 3.4.3.3. In the last two and a half years, puparia were found at only 8% of sampling sites and these were highly fragmented, as in Figure 3.4.3.4. Overall, a greater proportion of buried puparia were recovered, especially during the summer, with the surface puparia being the least likely to be recovered.

3.5 Discussion

The predominant blowfly species on the indoor/outdoor piglets was *Calliphora vicina*, the most common species found in the UK on corpses, especially in urban situations (Smith, 1986), although *Lucilia sericata* and Sarcophagidae were also present in the warmer season. In the more rural location of Kew Gardens, the predominant blowfly species was *Lucilia sericata*, which is well adapted to competition with other carrion-feeding species (Smith, 1986). The pairs of piglets were placed indoors and outdoors at different times of the year, including during cold periods, and it was temperature, rather than accessibility, which was the dominating factor in the decomposition of the piglet cadavers. Previous studies demonstrated a delay in oviposition of indoor cadavers, of 24 hours (Reibe & Madea, 2010) and as much as five days (Anderson, 2011), however in two out of five piglets studied here, indoor and outdoor oviposition occurred on the same day. In Run 1, oviposition took place on both piglets on the same day, even though it was mid-December, but the ambient temperature dropped below freezing over the next three days, resulting in the development of the larvae on the outdoor piglet being severely slowed down. In Run 3, carried out in May, oviposition took place on both indoor and outdoor piglets on the 2nd day of exposure, and both piglets decomposed at a similar rate, due to mean ambient temperature difference of only 4.3°C. In Runs 2 and 5, oviposition was delayed indoors by 10 and two days respectively. But in Run 4, oviposition was delayed outdoors by nine days due to ambient temperatures not rising above 10°C. Due to the buffering of indoor temperatures, these piglets tended to decompose faster than those outdoors, provided oviposition occurred on the same day. In the indoor/outdoor piglet experiments, the outdoor piglets did not show any marked increase in larval mass temperature above ambient temperatures, whereas some of the indoor piglet dataloggers showed a maximum temperature of up to 42°C.

PMI estimates are generally made using a few baseline studies in a controlled laboratory environment, and then extrapolating the data, but it is important to carry out field studies to validate these studies (Catts,

1992). The piglets placed outdoors in a rural environment also exhibited differences in attracting blowflies. In the 2nd experiment, two of the four piglets did not attract flies when they were first put out, although by Day 2 all four piglets had egg masses on them. The piglets also decomposed at slightly different rates, with some being reduced to bones after just ten days, whilst others still had soft tissue remaining on them after 28 days. The predominant species on these cadavers was *Lucilia sericata*, the larvae of which reached the pre-pupal stage in eight days in the first experiment, when mean temperature = 14.5°C, and six days in the second experiment, when mean temperature = 17°C. Although the first experiment was carried out in May, and the second in August, these temperatures are still relatively low, compared to the temperature range of most developmental laboratory studies. Anderson (2000) suggests that at 15.8°C *Lucilia sericata* reaches the pre-pupal stage in 233.7 hours, i.e. 9.7 days. Despite being small, the piglets at Kew Gardens showed significant increases in larval mass temperature, up to 42°C, close to their upper thermal limit, and 15°C above the ambient temperature, showing the importance of measuring both larval mass and ambient temperatures.

In the pupal longevity experiment, carried out in a typical Southern UK woodland, empty pupal cases of the common blowfly species *Calliphora vicina* were found to disintegrate over time, being more easily found and recovered in the first three and a half years after placement. Pupae placed on the surface were least likely to be found, whereas those which were buried or in the leaf/soil interface were more easily located. Season was also a contributing factor, as a greater number of puparia were recovered in the summer than in winter or autumn. In summer, buried larvae may be more easily found and recovered than in winter, due to the soil being dryer and falling away from the puparia, whereas when the soil was damp, it stuck to the puparia making them harder to see. The number of seasons passed is also important, as increased leaf litter may have been a factor in the difficulty of finding the surface-placed puparia. In this study, the pupae were of a known species; however, future studies should also consider the ease of identification of degraded pupal cases, because without accurate identification, a minPMI cannot be estimated.

CHAPTER 4

HUMAN DECOMPOSITION

4.1 Introduction

Forensic entomology is a fascinating area of science in which to carry out fundamental research, but ultimately the purpose of it is to produce data which can be used in forensic casework. Laboratory studies may be carried out to produce peer-reviewed publishable data which can be applied in forensic cases, and fieldwork studies may be carried out to ascertain the forensically important arthropod species composition in specific localities, environments and seasons, with pigs generally being utilised as a suitable substitute for humans.

If a scientist is to be employed in carrying out forensic casework, there is, however, no real substitute for a human body in decomposition studies when it comes to gaining experience. But crime scenes are highly protected areas, where the forensic entomologist is required to spend as short an amount of time as possible, to sample from the body non-invasively and without contaminating the scene or the body. Crime scene work is also relatively intermittent therefore the opportunity to gain experience of sampling from a human cadaver is rare. In addition, when a body is found the forensic entomologist, together with all the other forensic practitioners involved, only see a snapshot of the decomposition process. They do not see what happened prior to the body being found, such as the oviposition of flies and the distribution of larval masses on the body, and once the body is recovered from the scene it is removed to the mortuary, placed in a refrigerator and the decomposition process is halted. Slone and Gruner (2007) suggested that it is impractical to use human cadavers for blowfly development research, due to the lack of available replicates. However, the opportunity to observe the decomposition of humans and to sample the insect colonisers over an extended period is invaluable, both in terms of experience gained and in refining sampling techniques (Schoenly *et al.*, 2007).

It is, however, possible to study the process of human decomposition, from fresh to skeletal, at the Anthropological Research Facility (ARF) where human donor cadavers are used for scientific research. The Facility, which is affiliated with the Department of Anthropology, University of Tennessee, USA, is located on the outskirts of Knoxville, and was opened in 1980 when Dr William Bass, a Physical Anthropologist at the University and the first State Forensic Anthropologist of Tennessee, recognised the need for an outdoor laboratory to enable the study of decomposition of human cadavers. The three acre site is surrounded by two eight foot fences: an inner wooden fence and an outer metal fence topped with razor wire. Inside the fencing, the area is comprised of a small amount of flat gravelled areas set among mainly sloping wooded areas containing mature hardwood trees of oak, maple and black walnut.

The donor programme received its first donated body in 1981, and now receives over 100 body donations a year. Overall, 53% are family/friend donations, 26% are donated by medical examiners (ME) and 21% are self/pre-registered donors. Out of the 121 donors received last year (2010), 4% were ME donations and 31% were self-donors. There are currently over 2200 pre-registered donors [information supplied by Rebecca Wilson-Taylor, Assistant Coordinator of the Forensic Anthropology Center]. Donations are declined if the individual has an infectious disease such as HIV, hepatitis, tuberculosis or an antibiotic resistant infection such as MRSA.

When a body arrives at the site, all identifying personal objects are removed, such as clothing and jewellery, and each body is assigned an identifying number which is stamped onto metal tags and attached to one arm, one ankle and a wooden post in the ground close to where the body is placed, ensuring that the remains can be easily identified even if the skeleton is dislocated. Although all donated bodies are placed at the ARF, not all are suitable for use in research projects, such as those which have had a post-mortem carried out on them. Most bodies are laid directly on the ground under black plastic, which allows insects to gain easy access to them, and results in faster decomposition by creating a dark, moist environment for developing blowfly larvae. In some cases, bodies are decomposed in different situations, such as being buried in

graves or under concrete, submerged in water, or placed in cars. The bodies are left to decompose naturally for six months to a year, before the skeletal remains are gathered up, cleaned, inventoried, measured and various data collected. The remains are then accessioned into the William M. Bass Donated Skeletal Collection for future use in research, teaching and forensic casework. Currently the collection holds over 870 skeletons, predominantly Americans of European and African ancestry, and a smaller proportion of Hispanic ancestry, ranging in age at death from foetal up to 101 years old. The great value of this collection is that with each skeleton there is a file containing information on sex, age, race, height, weight (either measured or estimated), lifestyle, medical and dental history. In addition, most donor files contain an ante-mortem facial photograph of the person, so their skull can be used in future cranio-facial reconstructions.

Concerns that the Facility may suffer from “arthropod saturation” due to carcass enrichment were shown to be unfounded in a study in which pigs were placed both inside the Facility and at three sites outside it, at a distance of 700m, 6km and 40km (Shahid *et al.*, 2003).

In addition to sampling directly from the human cadavers, working at the ARF enables the opportunity to observe the dispersal of post-feeding Calliphorid larvae, which move off the body to find a place to pupariate (Greenberg, 1991). In an outdoor environment they will burrow into the soil or move under vegetation (Gennard, 2007). In an indoor environment, they will move under carpets and furniture. This means that when a forensic entomologist arrives at a crime scene to collect evidence, the oldest stages, which are the most important because they will give the minimum post mortem interval, may not be on the body, but may have already moved off it to pupariate. Large numbers of *Lucilia* spp. have been recorded moving 3.7m-4.6m and up to 6.4m from sheep carcasses (Cragg, 1955), although on concrete floors they have been observed dispersing up to 30m from the body (Green, 1951). Two blowfly species, *Calliphora vomitoria* and *Lucilia caesar*, were reported to disperse at night in order to minimise the possibility of predation (Kočárek, 2001). Tessmer & Meek (1996) suggested that post-feeding Calliphorid larvae preferentially disperse

towards the southeast during summer and spring, and towards the southeast and southwest during autumn. Studies have also suggested that movement off a body is random (Gomes & Von Zuben, 2005), although for some species larvae may follow a distinct trail left by previous larvae (Arnott & Turner, 2008).

Within a laboratory environment, larvae have been released within a confined space and therefore limited in the distance they can move: a 3m long run (Godoy *et al.*, 1995), 8.1m long channels (Greenberg, 1990), and 50cm diameter circular arenas (Gomes & von Zuben, 2005).

4.2 Insect succession and blowfly development

4.2.1 Aims

The aim of this study was to record the insect activity associated with the decomposition of a human cadaver from fresh through bloat, active and advanced decay, to the dry/skeletal stage, over a three week period. Although there were no replicates, this was seen as valuable experience into collecting insect evidence from a human cadaver over the period of decomposition from fresh to mummified, as a precursor to further studies. The behaviour of the most forensically important insects was monitored, including the rate and sites of oviposition of blowflies and development of larval masses on the body.

4.2.2 Materials and methods

4.2.2.1 Study site

The site used was the Anthropological Research Facility, Knoxville, Tennessee, USA, details of which are outlined in the Introduction.

4.2.2.2 Experimental model

The donated human cadaver was a 73 year old female weighing 91 kilos, whose body had previously been donated and stored in a mortuary cold store. There was a small open wound, approximately 1cm in length, on the front right side of the neck where a medical tube had been previously been inserted.

4.2.2.3 Placement of cadaver

The body was laid naked on the ground with three Tinytag Plus datalogger probes inserted into the mouth, rectum and torso (from the dorsal side) (Figure 4.2.3.1). A fourth datalogger was inserted under the right armpit on Day 3. A Tinytag Plus ambient datalogger was placed approximately 3 metres from the body. All the dataloggers had previously been set to record the temperature every 15 minutes.

4.2.2.4 Sampling procedure

Twice a day, at approximately 08:30 and 15:30, the cadaver was visited. At each visit, a TN1 non-contact infrared thermometer was used to record surface body temperatures and the surface temperature of any conspicuous Calliphorid larval masses. Any observations made were noted down, and photographs were taken. Larval samples of at least 100 individuals were also collected at each visit from each discrete larval mass, killed by immersion in freshly boiled water, and then preserved in 70% ethanol. Adult and larval beetles were collected by hand and using pitfall traps and also preserved in 70% ethanol. Larvae were later measured for analysis, choosing 20 from each sample, generally the largest but ensuring they were of the same species.

4.2.3 Results

4.2.3.1 Oviposition

Within 5 minutes of being laid out (Figure 4.2.3.1), the body had blowflies buzzing around it and landing on the face. By the second site visit, four hours later, a huge egg mass was protruding from the nose (Figure 4.2.3.2). Smaller egg masses were also laid in the folds of the ear, inside the left eye, the mouth and in the hairline. On Day 2, egg masses were laid on the scalp and in the neck wound (Figure 4.2.3.3). On Day 3, flies began ovipositing in the genital area (Figure 4.2.3.4) and under a leaf stuck to the left thigh (Figure 4.2.3.5). On Day 4, eggs were still being laid, but on other areas of the body less suitable for oviposition, such as the soles of the feet (Figure 4.2.3.6).

Figure 4.2.3.1 Day 1: Body laid out with internal temperature probes in place

Figure 4.2.3.2 Day 1: Large egg mass protruding from the nose after 4 hours

Figure 4.2.3.3 Day 2: Egg masses on the scalp and in the neck wound

Figure 4.2.3.4 Day 3: Eggs laid in the genital area

Figure 4.2.3.5 Day 3: Eggs laid under a leaf stuck onto the side of the leg

Figure 4.2.3.6 Day 4: Eggs laid on the sole of the right foot

4.2.3.2 Larval development

On the night of Day 1, there was a severe storm and torrential rain. The following morning, Day 2, the eggs forming the large egg mass on the face had hatched into 1st instar larvae, although many of them had been washed onto the ground (Figure 4.2.3.3). By the following morning, Day 3, the larvae on the face had moulted into 2nd instar larvae and were covering the face (Figure 4.2.3.7), and by that afternoon they had developed into 3rd instar and had formed a large larval mass (Figure 4.2.3.8) on the face and neck, causing sloughing off of the skin. By Day 4, the body was in full bloat, the head was blackened from bacterial decomposition, and larval masses were observed down the sides of the body and in the genital area (Figure 4.2.3.9). By Day 5, the body was still fully bloated (Figure 4.2.3.10) [Note: the orange and pink colouration on the body was caused by dyes being used to colour the larvae in order to track their post-feeding dispersal]. On the morning of Day 5 there was still a large 3rd instar larval mass on the head, but by the afternoon the larvae had moved onto the ground and further down the neck area and the eye sockets were exposed. By Day 6, the skull was completely exposed and large larval masses were recorded down the sides of the body and in the genital region (Figure 4.2.3.11). By Day 7, a lot of decomposition fluids were running off the body onto the ground, in which larvae were feeding, and a few of the larvae had started to leave the body to pupariate (Figure 4.2.3.12). By Day 8, the body had deflated and a huge “maggot soup” had formed around the body (Figure 4.2.3.13), especially around the lower part of the body which was on a downhill slope, and although there were still larval masses around the sides of the body, a large number of larvae were leaving the body. The biggest larval dispersal was seen on Day 9 and for the next 5 days (discussed in more detail in Section 4.5). After Day 9 the body continued to deflate and started to dry out. By Day 16, the body was mummified, the majority of larvae had left the body to find a suitable pupariation site, and the “maggot soup” was replaced by a large stain (Figure 4.2.3.14). This was the last day that daily observations were made.

Figure 4.2.3.7 Day 3 (am): 2nd instar larvae covering the face

Figure 4.2.3.8 Day 3 (pm): 3rd instar larval mass on face and right neck

Figure 4.2.3.9 Day 4: Body is in bloat and there are extensive larval masses

Figure 4.2.3.10 Day 5: Body still in full bloat and larvae are mostly 3rd instar

Figure 4.2.3.11 Day 6: Body has begun to deflate and pitfall traps were dug

Figure 4.2.3.12 Day 7: Larval masses surrounding body in fluid

Figure 4.2.3.13 Day 8: Body surrounded by fluid containing feeding larvae

Figure 4.2.3.14 Day 16: Majority of larvae had left the body to puparia

Further photographs were taken intermittently by other people working at the site, and the last photos were taken on Day 82, by which time the remaining soft tissues were totally mummified (Figure 4.2.3.15) and the skull, neck and upper vertebrae were exposed (Figure 4.2.3.16).

Figure 4.2.3.15 Day 82: Mummified remains (Photo: Michelle Harvey)

Figure 4.2.3.16 Day 82: Exposed skull, neck & vertebrae (Photo: Michelle Harvey)

4.2.3.3 Post-mortem artefacts

On Day 3 only, black ants were observed feeding on the legs and arms of the body (Figure 4.2.3.17) causing wounds which could be mistaken for chemical or cigarette burns (Figure 4.2.3.18).

Figure 4.2.3.17 Day 3: Black ants feeding on the arm

Figure 4.2.3.18 Day 3: Post-mortem wound on leg caused by feeding ants

4.2.3.4 Arthropod species

Table 4.2.3.1 lists the species of arthropod, from families Diptera, Coleoptera, Hymenoptera, Lepidoptera and Isoptera, collected manually from the body over the three week study. They were not collected intensively by hand, so neither chronological nor quantitative data is presented.

Table 4.2.3.1 Adult arthropod species collected from the body during the three week experimental period

Order	Family	Species
Diptera	Calliphoridae	*Cochliomyia macellaria*
		Phormia regina
	Muscidae	*Hydrotaea aenescens*
	Stratiomyidae	*Ptecticus trivittatus*
		Hermetia illucens
	Culicidae	*Culex pipiens*
	Piophilidae	*Stearibia nigriceps*
		Protopiophila latipes
Coleoptera	Silphidae	*Necrophila americana*
		Nicrophorus orbicollis
		Necrodes surinamensis
	Cleridae	*Necrobia rufipes*
		Necrobia ruficollis
	Histeridae	*Hister* sp.
	Staphylinidae	*Creophilus maxillosus*
	Dermestidae?	*Dermestes* sp.
Hymenoptera	Formicidae	Unidentified sp.
	Vespidae	*Vespa germanica*
		Dolichovespula maculata
	Myrmicidae	*Crematogaster lineolata*
Lepidoptera	Sphingidae	*Amphion floridensis*
	Nymphalidae	*Asterocamper celtis*
Isopoda	Armadillidiidae	Unidentified sp.

4.2.3.5 Larval development

Diptera larvae were collected from the body and identified as *Phormia regina*. Figure 4.2.3.19 shows the mean larval lengths of samples (for each sample n=20) taken from different larval masses on the body from Day 2 to Day 9 of the experiment.

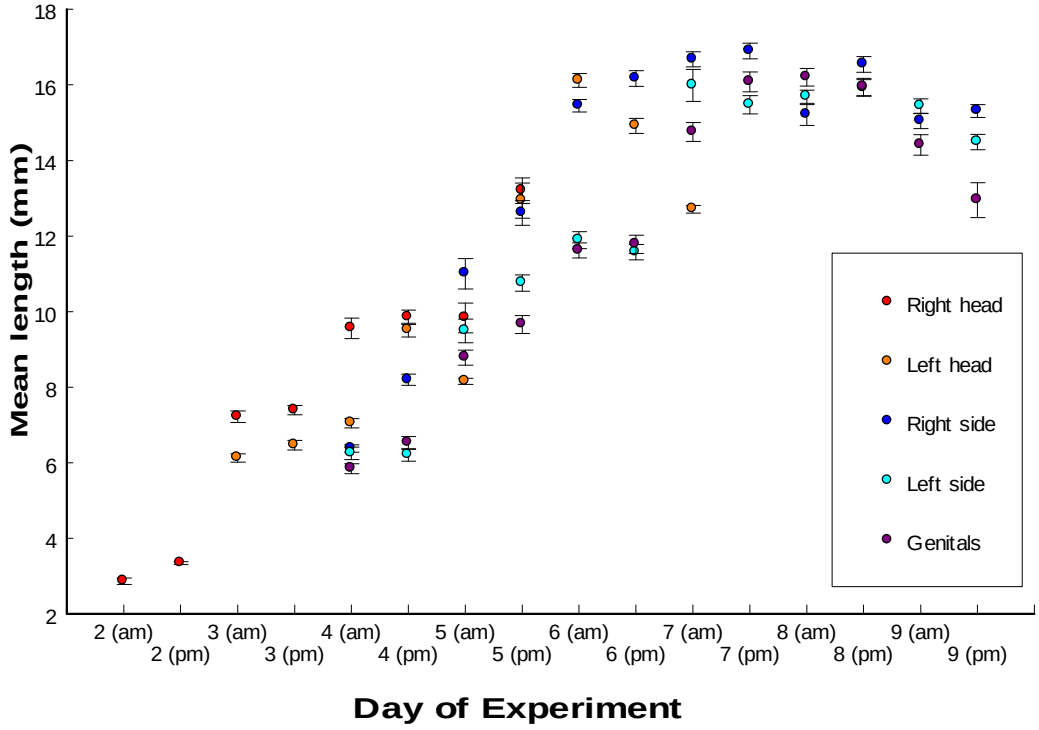


Figure 4.2.3.19 Larval lengths of *Phormia regina* collected from different areas of the cadaver (n=20 for each sample)

The larvae first started developing on the right side of the head but quickly spread to the left side of the head as a larval mass formed. Development on the left and right sides of the body and on the genitals was delayed by about two days because of the similar delay in oviposition. The larvae on all regions of the body developed at the same rate, reaching the maximum length of ~17mms before constricting in size after 6-8 days in order to form puparia.

4.2.3.6 Temperatures

At the start of the experiment, the back and rectal temperatures were 15°C because the body had come out of a cold store (Figure 4.2.3.20). The first area to show an increase in temperature was in the mouth where the first eggs were laid and therefore where the first larval mass developed. The temperature reached nearly 45°C on Day 5 and then rapidly dropped to around the same as the ambient as the skull became skeletonised and the larvae moved lower down the body into the neck and torso, where temperatures did not start to rise until Day 3. There was also a two day delay in oviposition in the genital region, which started on Day 3, so there was a subsequent delay in increased temperatures in this area. As the larval masses developed, they continued to increase in temperature, regardless of the fluctuating ambient temperatures, so at some stages the larval masses were 20°C above the ambient temperature, and there was a marked contrast between the clearly diurnal fluctuations of the ambient temperature and the mainly smoothly increasing body temperature. Once the larvae reached full size on Day 7 (Figure 4.2.3.19), the larval mass temperatures remained higher than the ambient temperatures but they became diurnally synchronised with it, rising during the day and decreasing at night.

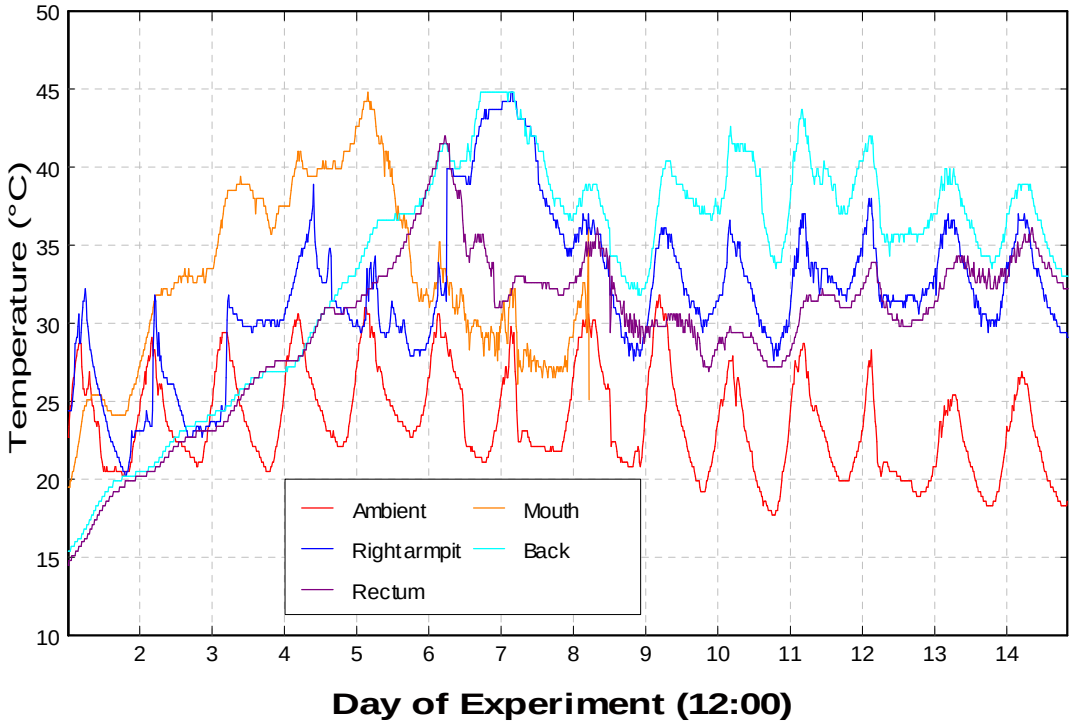


Figure 4.2.3.20 Internal body temperatures, recorded with datalogger probes in mouth,right armpit, back and rectum, plus ambient temperature, from Day 1-15

The internal body temperatures over ambient temperature are plotted in Figure 4.2.3.21. They show that the greatest difference was during the night-time, when ambient temperatures were coolest but the larval mass temperatures retained their heat. After midday however, the difference between body temperature and ambient temperature was at its lowest, when the ambient temperatures were at their highest.

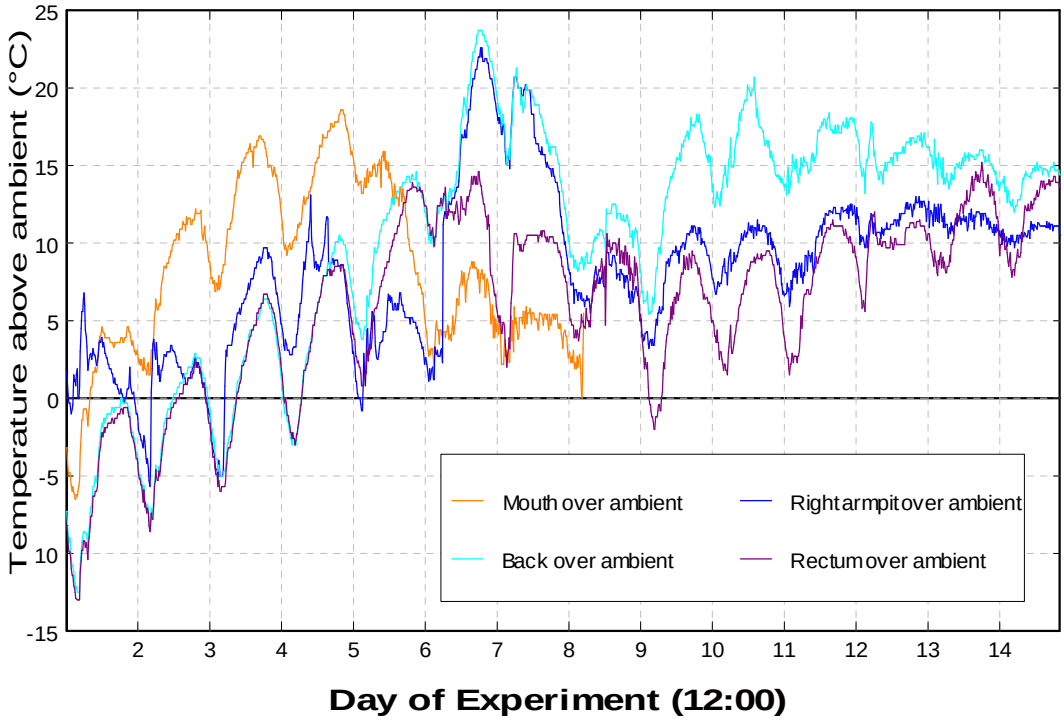


Figure 4.2.3.21 Internal body temperatures above ambient, recorded with datalogger probes in mouth,right armpit, back and rectum, plus ambient temperature, from Day 1-15

The larval mass temperatures show a similar pattern (Figure 4.2.3.22) where the temperatures of the head rose first, reaching a maximum of 42°C before dropping on the afternoon of Day 6. The increased temperature on the left and right side of the body and the genitals lagged behind the head region by at least one day, due to the delay in oviposition.

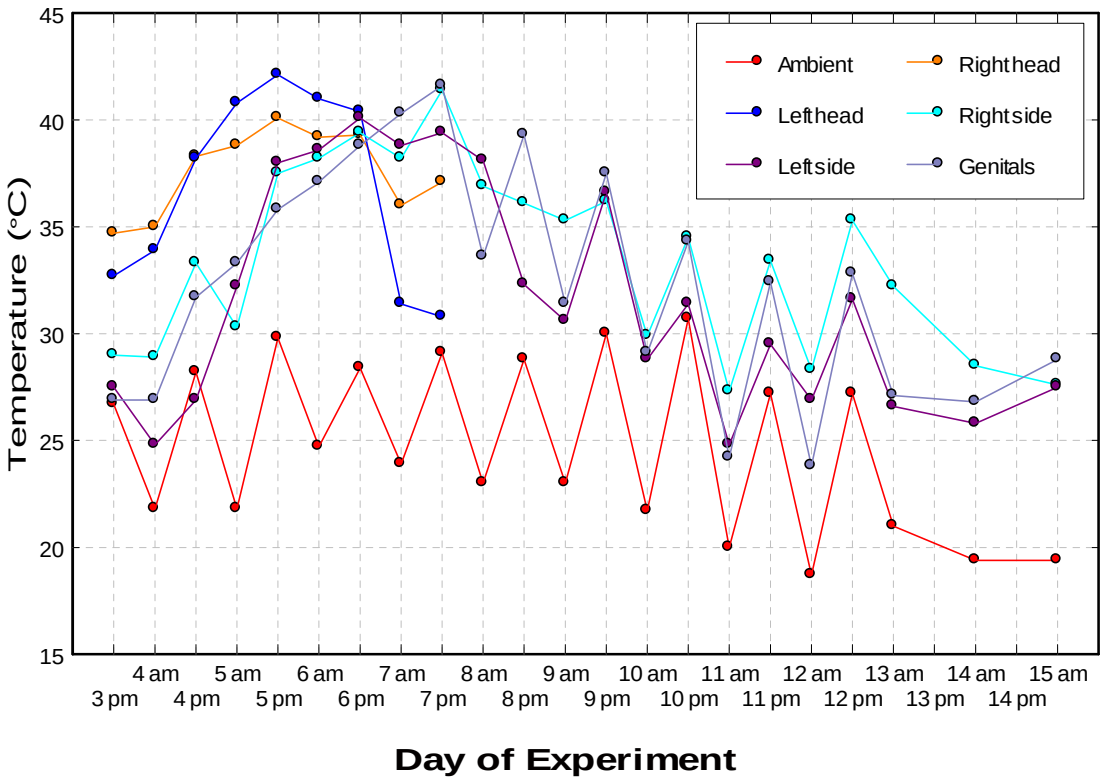


Figure 4.2.3.22 Maximum temperatures of larval masses, taken with infrared thermometer, from right head, left head, right side, left side and genitals, morning and afternoon from Day 3-15

4.3 Effect of reduced accessibility on insect succession and larval development

4.3.1 Aims

The aim of this study was to observe the insect activity, larval mass temperatures and decomposition of a cadaver in an environment with reduced accessibility to insects. Although there were no replicates, the body described in the previous section was acting as a control subject. The hypothesis was that there would be a disruption to the normal insect succession, blowfly oviposition and larval development. Body internal temperatures compared to ambient temperatures and decomposition rate would all be studied. The resulting information would be compared with studies undertaken elsewhere, but not discussed here, on cadaver decomposition in cars.

4.3.2 Materials and methods

4.3.2.1 Study site

The site used was the Anthropological Research Facility, Knoxville, Tennessee, USA, details of which are outlined in the Introduction.

4.3.2.2 Experimental model

The human cadaver used was a 72 year old female weighing 68 kilos, whose body had been donated for scientific research at the Facility. The body was intact and there were no external wounds visible.

4.3.2.3 Placement of cadaver

The cadaver was placed naked in a brand new large blue plastic wheelie bin, bent at the hips, with the feet and head pointing downwards. Three Tinytag Plus dataloggers were set to record temperatures every 15 minutes. One datalogger was attached to a tree a few feet above the bin to record ambient temperatures. Two of the dataloggers had probes attached which were pushed

through small holes drilled in the lid of the bin – one was attached to the inside of the bin lid to record ambient temperatures inside the bin, while the other was inserted into the rectum of the body to record internal body temperatures. Tape was used to seal the holes in the bin to ensure no insects could enter through them.

4.3.2.4 Sampling procedure

The cadaver was visited and observed once a day, at approximately 08:30, when the lid was raised for a short period. This was the coolest time of the day when insect activity was limited and care was taken to ensure that no insects entered the bin when the lid was raised. The presence of any insects was noted and sampled by hand using long forceps, and photographs were taken. Larvae were killed by immersion in freshly boiled water and all samples were stored in 70% ethanol. The study was carried out at the same time as that described in Section 4.2.

4.3.3 Results

4.3.3.1 Oviposition and larval development

From Day 1 to Day 6, no insects were observed inside the bin. The first flies to gain access inside the bin (4.3.3.1) were Phorid flies, *Megaselia scalaris*, which were first seen on the body on Day 6 (Figure 4.3.3.2). By Day 9, numerous larvae of this species were crawling over the surface of the body and the inside surfaces of the bin (Figure 4.3.3.3), but no larval masses were forming. On Day 10 the first pupae of *Megaselia scalaris* were collected from the outer rim of the bin (Figure 4.3.3.4). The first Calliphorid flies were seen landing on the outside of the bin around the rim of the lid on Day 8 (Figure 4.3.3.5), and adults and 1st instar larvae were first collected from inside the bin on Day 9. By Day 10, predacious beetles had also gained access into the bin, including *Necrobia rufipes* and *Nicrophorus orbicollis*. By Day 13, Calliphorid larval masses had started to form in the crevices of the body (Figure 4.3.3.6) and larvae were crawling up the inside of the bin in order to escape. A number of dead Calliphorids were found inside the bin (14 on Day 12). Day 16 was the last day

of data sampling, but further insect samples and photographs were taken by other people. It appears from photographs that the body started to mummify by Day 26 (Figure 4.3.3.7), and by Day 82, large numbers of *Fannia* larvae were inside the bin (Figure 4.3.3.8). A hard crust started to form on the exposed surface of the body (Figure 4.3.3.9), while the whole body sank >50% lower into the bin (Figure 4.3.3.10). When the remains of the body were emptied out of the bin after 263 days, the upper surface was a hard crust (Figure 4.3.3.11), while underneath the head and shoulders were intact, and much fluid was present (Figure 4.3.3.12).

Figure 4.3.3.1 Day 1: The bin in situ (arrow shows the level of the body)

Figure 4.3.3.2 Day 6: Phorids, the first flies to access the body inside the bin

Figure 4.3.3.3 Day 8: Phorid larvae on the body inside the bin

Figure 4.3.3.4 Day 10:Phorid pupae on outside of the bin

Figure 4.3.3.5 Day 11:Diptera on the outside rim of the bin lid

Figure 4.3.3.6 Day 13: Calliphorid larvae on the body inside the bin

Figure 4.3.3.7 Day 26: The body had begun to mummify (Photo: Murray Marks)

Figure 4.3.3.8 Day 81: A crust started to form on top (Photo: Michelle Harvey)

Figure 4.3.3.9 Day 133: Advanced mummification (Photo: Murray Marks)

Figure 4.3.3.10 Day 263: The body had sunk >50% down from original depth

Figure 4.3.3.11 Day 263: A hard crust had formed on top of the body in the bin

Figure 4.3.3.12 Day 263: Remains of soft tissue when the bin was emptied

4.3.3.2 Temperatures

The external ambient temperatures fluctuated diurnally, ranging from a minimum of 18°C on the night of Day 10 to a maximum of 33°C on Day 9 (Figure 4.3.3.13). The internal ambient bin temperatures closely followed the external ambient temperatures although they lagged behind them a little and rose above them each day. The rectal temperatures also followed the same pattern, lagging behind both the external ambient and the internal bin temperatures. Neither the internal bin temperatures nor the rectal temperatures decreased as low as the ambient temperatures overnight. When the ambient temperatures rose during the day, however, the internal bin temperatures and the rectal temperatures increased over and above the ambient temperatures. From Day 10 onwards, the rectal temperatures remained significantly higher than the ambient temperatures overnight, and on Days 13 and 14 the rectal temperatures remained high, reaching 42°C on Day 14.

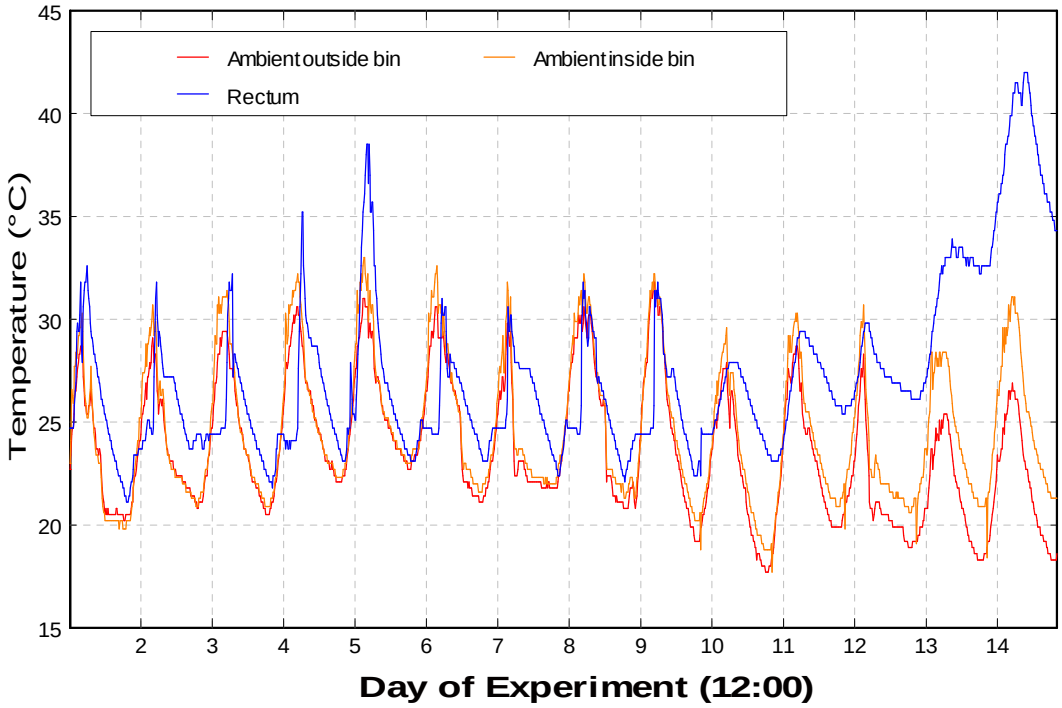


Figure 4.3.3.13 Datalogger temperatures recorded for ambient outside the bin, ambient inside the bin and cadaver rectum temperature inside the bin

The temperature difference between external ambient temperatures and both the internal bin temperatures and the rectal temperatures are plotted in Figure 4.3.3.14. They show that the internal bin temperatures remained higher than the external temperatures during the day, but that during the night they were

about the same. The daily downward blips in the inside bin temperature, most clearly seen from Day 10 onwards, occurred when the bin lid was raised each morning to carry out sampling, causing the internal temperature of the bin to briefly drop to that of the external ambient temperature.

From Day 1 until Day 10, the rectal temperatures remained below the ambient temperatures during the day, but did not reach the lower overnight temperatures. From Days 10 to 12, the highest internal body temperatures were just below the highest ambient and bin temperatures, but were buffered against the lower temperatures. On Days 14 and 15, the internal body temperatures were significantly raised to 15ºC higher than the internal ambient bin temperature and 19ºC above the external ambient temperature.

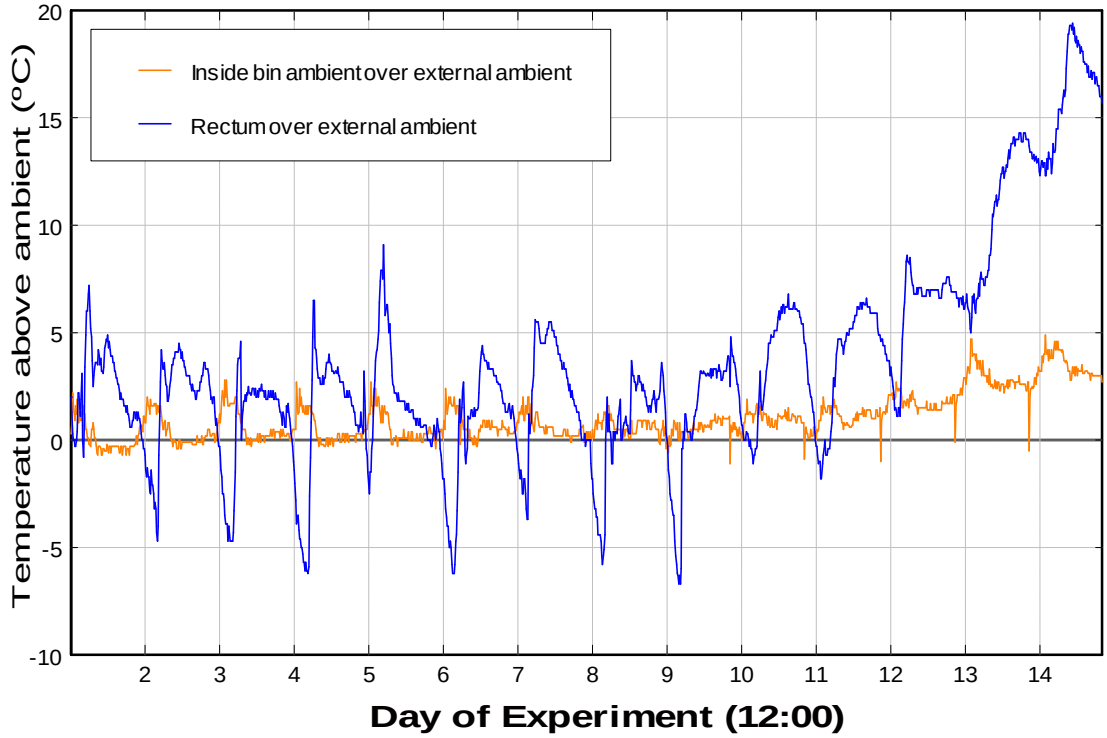


Figure 4.3.3.14 Datalogger temperatures above ambient for ambient temperature inside the bin and the rectal temperature inside the body inside the bin

4.4 Thermal dynamics of larval masses on a human cadaver

4.4.1 Aims

The aims of this study were to use thermal imaging to investigate the dynamics of larval masses on a human cadaver, from fresh through to dry/skeletal. In addition, the temperatures recorded by thermal imaging were compared with he ambient temperature. Further aims of the study were to determine whether there is a correlation between size of larval mass and temperatures generated, the hypothesis being that larger larval masses will generate a higher temperature. Heat retention and loss in larval masses was also investigated.

4.4.2 Materials and methods

4.4.2.1 Study site

The site used was the Anthropological Research Facility, Knoxville, Tennessee, USA, details of which are outlined in the Introduction.

4.4.2.2 Experimental model

The human cadaver used was an 87 year old female weighing 84 kilos, whose body had been donated for scientific research at the Facility. The body was intact and there were no external wounds visible.

Figure 4.4.2.1 Cadaver laid out

4.4.2.3 Placement of cadaver

The body was laid naked on the ground with three Tinytag Plus datalogger probes inserted into the oral cavity, rectum and torso (from the dorsal side) (Figure 4.4.2.1). A Tinytag Plus ambient datalogger was placed approximately 5 metres from the body. All four dataloggers had previously been set to record the temperature every 15 minutes.

4.4.2.4 Sampling procedure

A FLIR Systems ThermaCAM™ P65 thermal imaging camera was hired for a period of ten days. Twice a day, at approximately 08:30 and 15:30, the cadaver was visited. At each visit, a TN1 non-contact infrared thermometer was used to record surface body temperatures and the surface temperature of any conspicuous Calliphorid larval masses. Thermal images were taken twice daily, early morning and mid afternoon, of all major areas of the bodies, and any visible larval masses. Thermal images were also taken using a time-lapse setting, for a period of up to 10 minutes. Regular still photographs were taken of all the larval masses, some of which were photographed with a tape measure draped across them to estimate size. Any observations regarding the stage of decomposition and developmental stage of the larvae were noted.

4.4.3 Results

4.4.3.1 Thermal images

Figures 4.4.3.1 to 4.4.3.7 show the thermal images and still photographs from the body over a 4 day period, from Day 2 of being laid out, to Day 5. Maximum larval mass temperature and ambient temperature are also indicated. Figures 4.4.3.4 and 4.4.3.5 also include graphs of the temperature along the indicator line across the thermal image. The scale on each photograph are slightly different, because when the camera takes the image, it automatically chooses the most appropriate temperature range. Thus the range on each scale depends on the temperatures recorded in that particular image.

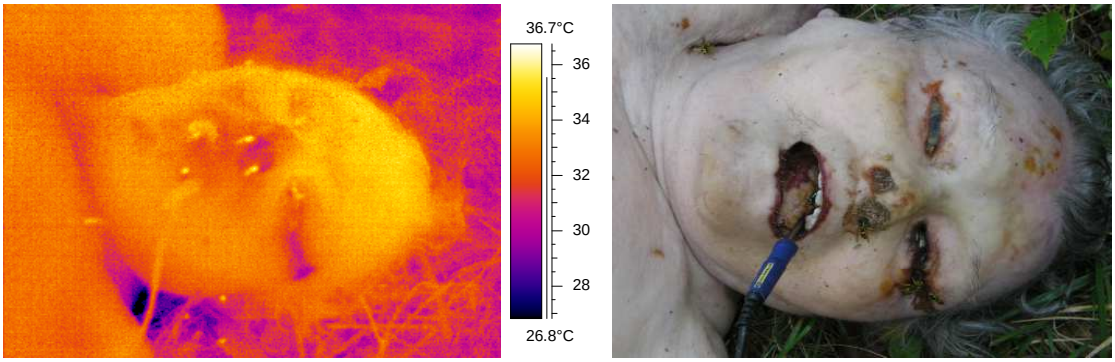


Figure 4.4.3.1 Day 2 pm, temps 34°C (maximum thermal) and 36°C (ambient). The bright spots in the thermal image in are adult flies visiting the body, therefore the maximum body temperature for analysis is noted as that inside the mouth.

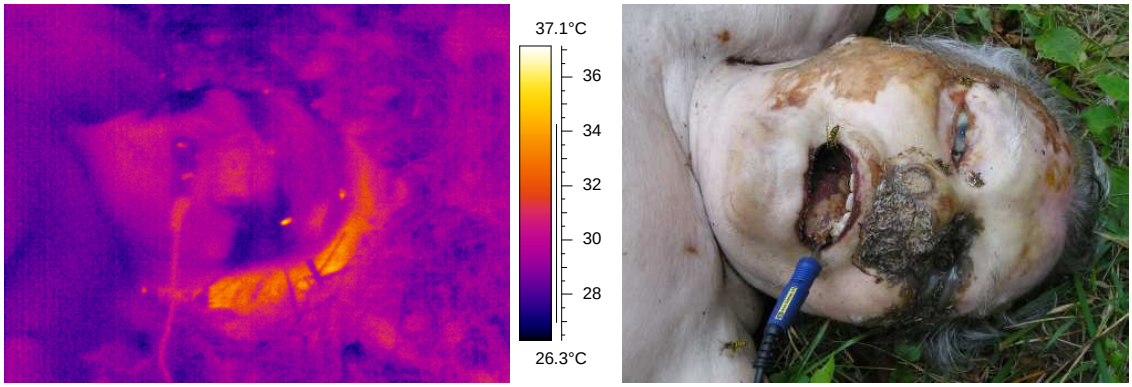


Figure 4.4.3.2 Day 3 am, temps 36°C (maximum thermal) and 30°C (ambient)

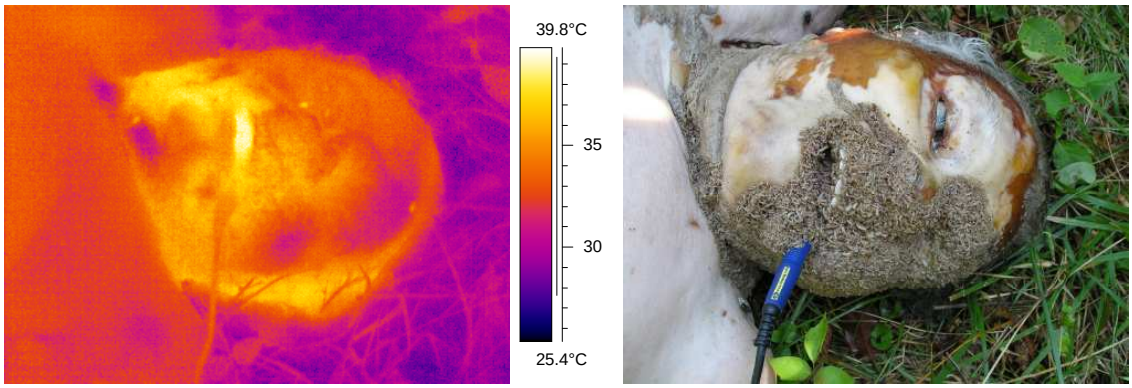


Figure 4.4.3.3 Day 3 pm, temps 39°C (maximum thermal) and 31°C (ambient)

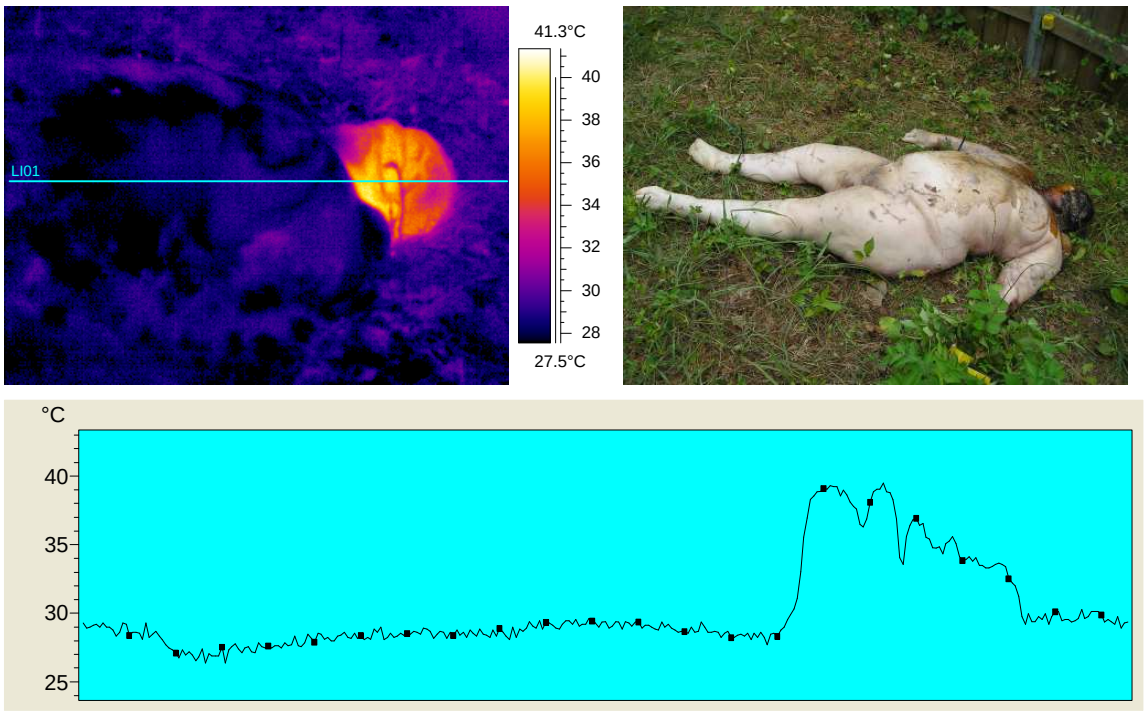


Figure 4.4.3.4 Day 4 am, temps 40°C (maximum thermal) and 28°C (ambient). The line across the thermal image is represented on the graph, with temperature (°C) on the y-axis, and the line LI01 graphically represented.

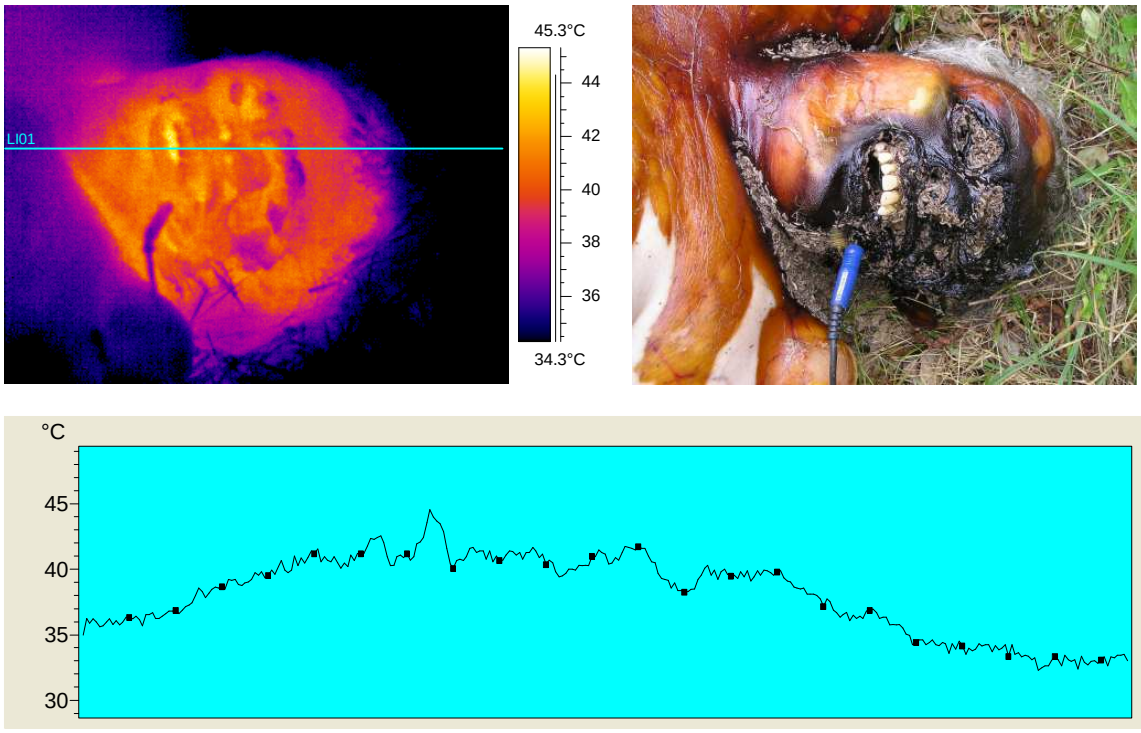


Figure 4.4.3.5 Day 4 pm, temps 45°C (maximum thermal) and 30°C (ambient). The line across the thermal image is represented on the graph, with temperature (°C) on the y-axis, and the line LI01 graphically represented.

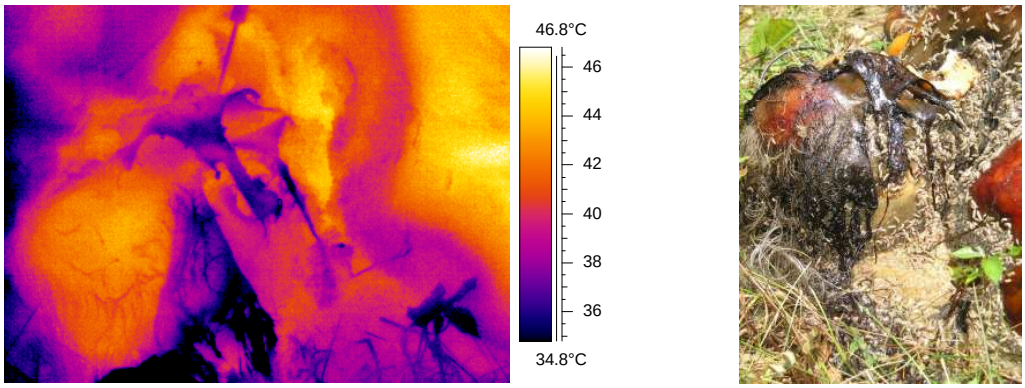


Figure 4.4.3.6 Day 5 am, temps 46°C (maximum thermal) and 38°C (ambient)

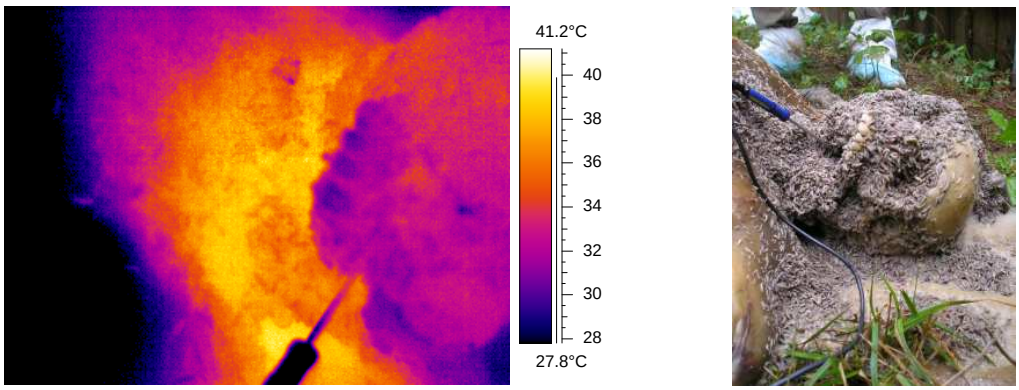


Figure 4.4.3.7 Day 5 pm, temps 40°C (maximum thermal) and 23°C (ambient)

On the morning of Day 3, the hottest area was around the back of the head rather than in the facial orifices (Figure 4.4.3.2), however by the afternoon of Day 3, the larval mass in the mouth had the highest temperature recorded on the thermal image (Figure 4.4.3.3), even though there was a large larval mass visible in the photograph on the left side of the face.

Figure 4.4.3.4 was taken a slight distance from the body, showing the entire torso and head, and shows clearly that on Day 3 the larval masses were focussed in the head region, whereas the rest of the body was the same temperature as the ground on which the body was lying. This was also shown by the graph below the images, which indicates the temperature of the torso was around 28°C i.e. the same as the ambient temperature. The same graph also shows a marked increase in temperature along the head of 40°C.

Figure 4.4.3.5 again shows that the highest temperature on Day 4 was 45°C inside the mouth, even though there were larval masses visible in the eye sockets and nose, and the temperature increase in the mouth is also indicated in the graph below. By Day 5, larval masses had started to spread through the neck and upper torso, and the photograph taken in the morning shows bright sunshine on the body (Figure 4.4.3.6). By the afternoon of the same day, the body was in shadow and although there were extensive larval masses over the face, the hottest area was actually in the neck, as indicated by the thermal image (Figure 4.4.3.7).

4.4.3.2 *Thermal and ambient temperatures*

Figure 4.4.3.8 shows the maximum larval mass temperature recorded in each thermal image against the ambient temperature recorded at the same time, and the difference between the two.

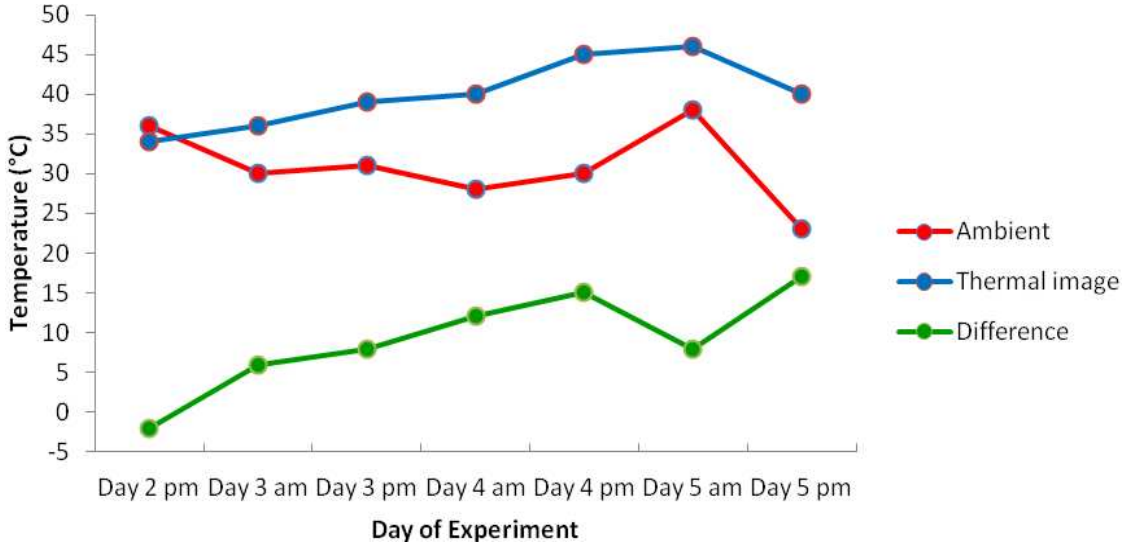


Figure 4.4.3.8 Larval mass and ambient temperature, and the difference between them

The maximum temperature recorded by the thermal images was marginally lower than the ambient temperature on Day 2, with both around 35°C. By the morning of Day 3, there was already a slight increase in the larval mass temperature, recorded by the thermal imaging camera. The difference between the maximum thermal image temperature and the ambient temperature continued to increase to 15°C on the afternoon of Day 4. There was a drop in difference between the thermal image temperature and the ambient temperature to 8°C on the morning of Day 5, even though the larval mass temperature was still increasing, because the ambient temperature increased by 8°C. By the afternoon of Day 5 the ambient temperature had dropped to 23°C, while the larval mass temperature also decreased by 5°C, but the larval mass temperature still remained 17°C higher than the ambient temperature.

4.4.3.3 Size of thermal masses

An example of one of the larval masses is shown in Figure 4.3.3.9, both by thermal image and with photographs. The tape measure positioned at two angles allowed an estimate of the area to the calculated. This was done for 19 different larval masses and the area (cm²) and maximum temperature (°C) is illustrated graphically in Figure 4.4.3.10. A regression line is drawn through the points, showing a significantly positive relationship between size (area) of larval mass and maximum mass temperature. There was, however, considerable variation and an increase in size of larval mass from 50-70cm² resulted in a temperature rise of just 4°C. The smaller the size of the larval mass, the greater the temperature variation (10°C), whereas the larger the area of larval mass, the smaller the temperature variation (3°C).

Figure 4.4.3.9 Larval mass with measuring tape, and corresponding thermal image

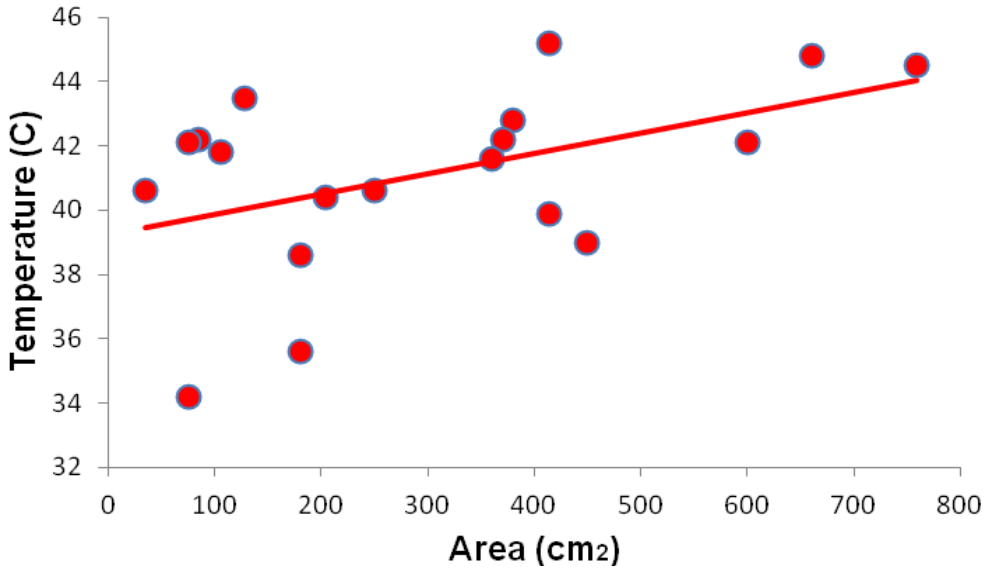


Figure 4.4.3.10 Area (cms²) and maximum temp (°C) of 19 larval masses

4.4.3.4 Heat retention of thermal masses

The larval mass in Figure 4.4.3.11 was already exposed to the environment and was filmed using time-lapse photography. 55 images were taken over a period of 5 minutes i.e. approximately 1 image every 5.5 seconds. During that time the minimum temperature ranged from 29.5°C-32.2°C and the maximum temperature ranged from 33.3°C-34.3°C along the line shown in Figure 4.4.3.12 (also in Figure 4.4.3.11) with an overall range of 4.8°C across the larval mass.

Figure 4.4.3.11 Thermal mass

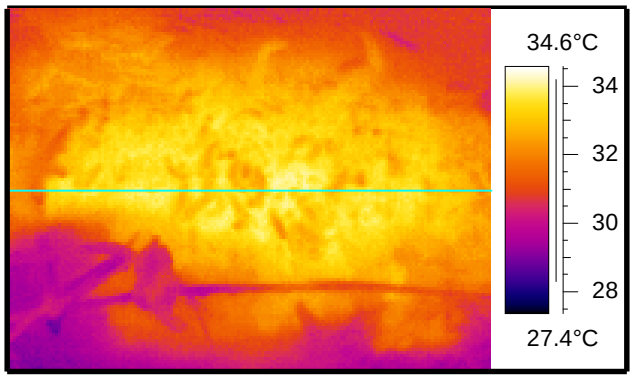


Figure 4.4.3.12 Thermal image

4.4.3.5 Heat loss of thermal masses

The body in Figure 4.4.3.13 was covered with black plastic, but on uncovering it, the larvae quickly dispersed. The six thermal images taken over a period of 1 minute 40 seconds show a rapid decrease in temperature (Figure 4.4.3.14). The area indicated by the blue circle in the first thermal image decreased in temperature from a range of 40°C-43°C to a range of 36°C-40°C during that time, an overall decrease of 3°C-4°C during the 1 minute 40 second period.

Figure 4.4.3.13 Cadaver after being uncovered from black plastic sheeting

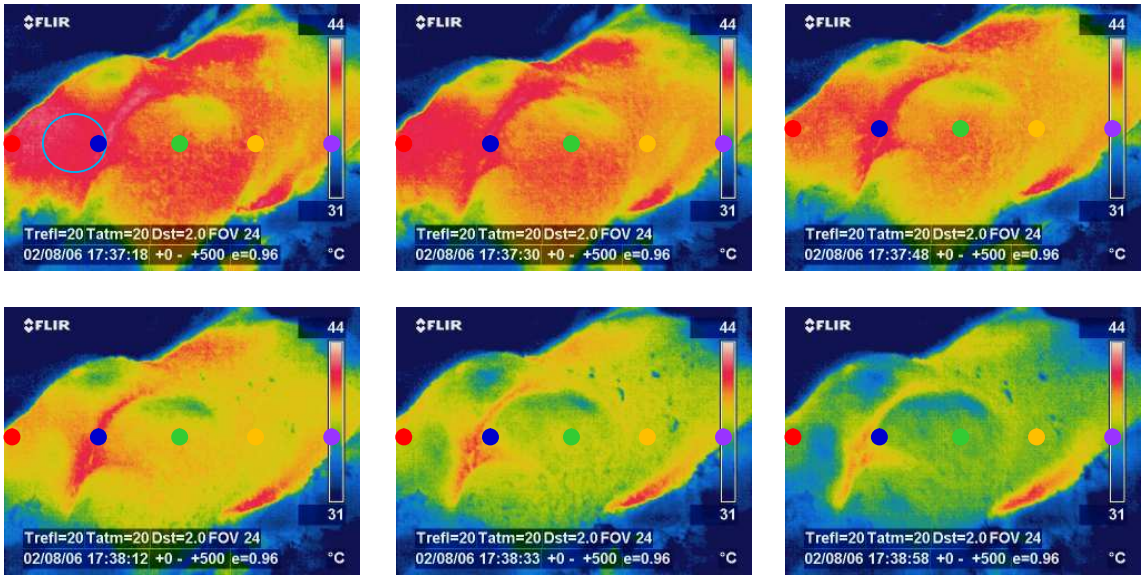


Figure 4.4.3.14 Thermal images of cadaver taken with a FLIR Systems ThermaCAM™ P65 camera over a period of 1 minute 40 seconds after black plastic sheeting was removed. The temperature at each of the coloured spots is shown in Figure 4.4.3.15.

The heat loss from these thermal images is shown graphically in Figure 4.4.3.15, using 5 spots at fixed intervals across the image, generated in FLIR software.

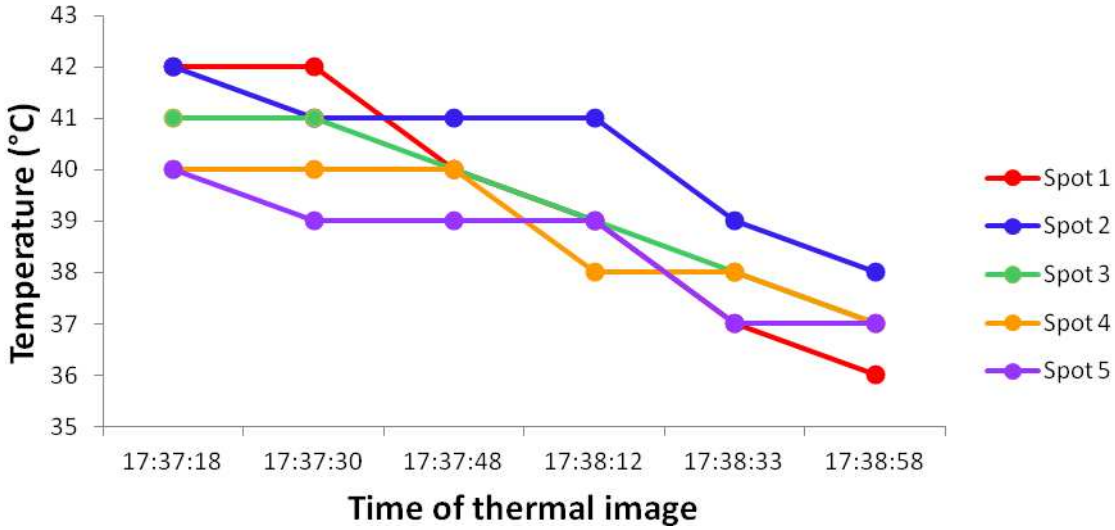


Figure 4.4.3.15 Heat loss from human cadaver over period of 1 minute 40 seconds after black plastic sheeting was removed, measured on thermal images in Figure 4.4.3.14 using spot analysis in FLIR software, illustrated using Spots 1 to 5 shown in Figure 4.4.3.14.

4.5 Dispersal behaviour of 3rd instar post-feeding blowfly larvae

4.5.1 Aims

The aim of this study was to make observations on the behaviour of post-feeding larvae dispersing from human cadavers and to determine whether their behaviour is consistent and therefore predictable. Specifically, the aim was to ascertain the direction of movement of larvae, distance travelled, duration of their dispersal and to observe whether they move independently of each other or if their behaviour is dependent on the behaviour of other larvae. The hypothesis is that larvae will move off the body downhill where possible, and in a single direction.

4.5.2 Materials and methods

4.5.2.1 Study site

The site used was the Anthropological Research Facility, Knoxville, Tennessee, USA, details of which are outlined in the Introduction.

4.5.2.2 Experimental models

Six donated human cadavers were observed during the period when post-feeding larvae were dispersing from them in order to pupariate. Details of the cadavers are given in Table 4.5.2.1, together with the month and year they were donated, their gender, age and weight. For Bodies A, C and D the age and weight were estimated. Bodies B, E and F were studied from the fresh stage for 15-16 days. Detailed observations are described in Section 4.2 for Body B. Body E was studied in detail but not included elsewhere in this report. Body F was studied in detail and is included in Chapter 5.

Table 4.5.2.1 Details of cadavers observed during the study [*estimated]

Cadaver	Month / year	Gender	Age (years)	Weight (kg)
Body A	July 2004	Male	55*	90*
Body B	July 2004	Female	73	91
Body C	July 2004	Female	65*	65*
Body D	July 2004	Female	85*	55*
Body E	July 2006	Female	87	84
Body F	July 2008	Male	59	68

4.5.2.3 Placement of cadavers

All cadavers were placed naked, lying on their backs, directly onto the ground and left uncovered in different areas of the Anthropological Research Facility, University of Tennessee, Knoxville, USA. Body E was laid in open ground and therefore fully exposed to direct sunlight. Bodies C and D were laid on gravel, exposed to direct sunlight for fewer daylight hours due to foliage overhead. Bodies A, B and F were more shaded due to being laid directly under large trees in more wooded areas.

4.5.2.4 Observation procedure

Each cadaver was visited and observed twice daily, at approximately 08:30 and 16:30. The movement of any larvae off the body was noted, and photographs were taken. The direction of movement was noted and the distance dispersed by the larvae from the body was measured by marking the furthest point at which the larvae could be found with a yellow flag, and then the distance between the flag and the navel of the body was measured. For standardisation this measurement (flag to navel) was used rather than from a specific larval mass e.g. in the head or groin, as it was not always possible to be sure of the exact area of the body from which the larvae had dispersed.

4.5.3 Results

Table 4.5.3.1 summarises the data observed from each cadaver. Figures 4.5.3.1 to 4.5.3.6 show each of the six cadavers at the stage where larval dispersal was taking place. Table 4.5.3.1 gives a summary of post-feeding larval movement. Descriptions of each cadaver and the larval dispersal are given in Sections 4.5.3.1 to 4.5.3.6. Variation in larval dispersal was recorded in each case.

Table 4.5.3.1 Summary of post-feeding larval dispersal

Cadaver	Dispersal start	No. of days	Direction	Max distance
Body A	<Day 11	Unknown	NE, NW, SE, SW	620cm
Body B	Day 7	7 days	Mainly NW	690cm
Body C	Day 10	3+ days	Entirely NE	Min 180cm
Body D	Day 7	7 days	Entirely NE	1120cm
Body E	Day 7	3 days	Mainly SW	Unknown
Body F	Day 8	5 days	NE, NW, SE, SW	400cm

4.5.3.1 Body A (Figure 4.5.3.1)

This cadaver was a victim of a road accident and had undergone a post-mortem. He therefore had a large Y-wound in the abdomen and it is likely that a large number of blowfly eggs would have been laid in this area, resulting in a large abdominal larval mass. The body had been laid out 11 days before this study began, and was already in an advanced stage of decomposition. There was a large 3rd instar larval mass still present and feeding in the genital region. Post-feeding larvae were already dispersing, however, and had probably been leaving the body on previous days, so the total number of days of larval dispersal is unknown. The larvae were observed leaving the body in four directions: 360cm northeast, 520cm northwest, 620cm southeast and 420cm southwest (mean = 480cm).

4.5.3.2 Body B (Figure 4.5.3.2)

This cadaver was closely observed throughout the decomposition process, details of which are outlined in Section 4.2. On Day 7, the first larvae started moving off the body. Because they were at the head end, these larvae moved away from this area, mainly towards a tree approximately 30cm from the left shoulder, in a south/southwest direction, where they buried down into the soil. Migrations off the body were observed on seven consecutive days, with relatively small numbers of larvae on the first and last of the seven days, and much larger numbers of larvae on the second to sixth days. Direct movement off the body was observed only in the early morning, at around 08:30 (Figure 4.5.3.7). By each afternoon, around 16:30 (Figure 4.5.3.8), although some larvae may still have been visible away from the body and above the soil, no persistent movement off the body was observed. Although a small number of larvae were observed moving in different directions, the vast majority moved in

a northwest direction, which was also on a downhill slope. In each of these consecutive migrations over seven days in the northwest downhill direction, the larvae were observed to follow the same narrow route away from the body, before reaching an obstacle in the form of a fallen tree, from where they then spread over a large area, although a large number of them continued to move around the obstacle and go a further 1m the other side of it. The maximum distances measured for dispersal of the larvae from the body were 690cm west and 530cm northwest (mean = 610cm).

4.5.3.3 Body C (Figure 4.5.3.3)

This cadaver had extensive mould on the face, having been kept in the mortuary refrigerator for a number of weeks. Blowflies were not immediately attracted to it, and therefore oviposition was delayed by a day and there were few egg masses. Larval masses did, however, form and dispersal began on Day 10 after it was laid out, and continued for another two days when the study period came to an end and no more observations could be made. The larvae all moved off the body in a northeast direction in two separate tracks from one side of the body, down a steep gravel slope to a wooden fence situated approximately 180cm from the body. It was not possible to determine whether the larvae stopped there or moved under the fence to the other side to pupate.

4.5.3.4 Body D (Figure 4.5.3.4)

This cadaver was laid out on the same day as Body C, and larvae started moving off the body on Day 7. The dispersal took place over a period of seven days, but mainly on Days 9 and 10. All the larvae moved from one side of the body in a northeast direction, following two trails. One day they followed one trail, the next day the other trail, and thereafter both trails. Both trails were narrow, and lead directly to a line of concrete blocks, at which point the majority of larvae turned 90º and moved along the edge of the concrete obstruction, before spreading out across the gravel. A few larvae moved under and even over the concrete blocks (Figure 4.5.3.9). Larvae were observed to move at least 1120cm from the body, both up and downhill.

4.5.3.5 Body E (Figure 4.5.3.5)

This cadaver was studied in detail in July 2006, but no further details are included in this report. The body was placed in an area of open grassland. During the hottest time of the day, i.e. mid-afternoon, the surface temperature of the body was often measured as being higher than the larval masses, which would have died at temperatures of that magnitude. Larval migration took place on Days 7 to 9, radiating out from the body in all directions, but mainly in a southwest direction. Because they were dispersing amongst grass (Figure 4.5.3.10), it was not possible to measure the distances moved by the larvae.

4.5.3.6 Body F (Figure 4.5.3.6)

This cadaver was studied in detail in July 2008. Larvae were dispersing from the body between Days 8 and 12, but the greatest larval movement from the body was from Days 10 to 12. Larvae radiated out from the body in all directions, moving uphill towards a gravel path (Figure 4.5.3.11). A number of them were observed to then turn back, move back towards the body and fall off a large rock to the same level on which the body was lying. On the morning of Day 11 there had been rainfall overnight and larvae were observed to be crawling up a nearby tree (Figure 4.5.3.12), located 100cm from the body. They were observed to move up to 400cm away from the body.

Figure 4.5.3.1 Body A

Figure 4.5.3.2 Body B

Figure 4.5.3.3 Body C

Figure 4.5.3.4 Body D

Figure 4.5.3.5 Body E

Figure 4.5.3.6 Body F

Figure 4.5.3.7 Larvae dispersing from Body B in the morning

Figure 4.5.3.8 Larvae from Body B burrowing in the soil in the afternoon

Figure 4.5.3.9 Larvae from Body D dispersing around concrete blocks

Figure 4.5.3.10 Larvae from Body E dispersing amongst grassland

Figure 4.5.3.11 Larvae from Body F dispersing across a gravel path

Figure 4.5.3.12 Larvae from Body F moving up a tree trunk after rainfall

4.6 Discussion

It has been suggested that the site in Knoxville, Tennessee may suffer from "arthropod saturation" due to "carcass enrichment", i.e. the regular exposure of human cadavers since 1981 would result in the area becoming dominated by the most tolerant or competitive species, thus altering species richness (Kempton, 1979), and in turn modifying the rate of cadaver decomposition. Using pig carcasses, Shahid *et al.* (2003) investigated this theory by placing the carcasses at the Anthropological Research Facility and at three other local "non-enriched" sites. They found no significant difference between the four sites in rate of carcass decay, numbers of sarcosaprophagous taxa or colonisation rate, and therefore concluded that the site is representative of the surrounding environment and therefore a suitable site for decomposition experiments to be carried out.

Within a few minutes of being exposed, the body on the ground was investigated by blowflies and oviposition began to take place in the facial orifices. The resulting egg mass protruding from the face was laid by a large number of individuals all ovipositing in the same area, referred to as communal oviposition, and may occur in order to minimise water loss (Barton Browne *et al*., 1969). An increase in eggs will also result in a larger larval mass, which in turn may result in increased heat and therefore faster development. Some Simulids lay eggs which release pheromones directly after oviposition to

encourage further oviposition on the same substrate by gravid females, called "aggregation pheromones". By Day 3, blowflies had started laying eggs in the genital region, under leaves stuck to the leg, and even on the base of the foot. By this time there was a large larval mass on the face and head, which made it unsuitable for further egg-laying. After the 5th day the body was in active decay, and therefore the chemicals being omitted may no longer have been attractive to the blowflies (Erzinçlioğlu, 1996).

The internal body temperatures of the cadaver on the ground were quite low at the start of the study, <10ºC, due to it having come straight from a local mortuary, where it was stored at approximately 4-5 ºC. The ambient temperatures in Tennessee at that time of year ranged from around 20ºC at night to 30ºC during the day, enabling the body to warm up within three days. Within two days of oviposition, however, the temperature in the mouth was already considerably higher than the ambient temperature, because the first egg masses were laid in this region, and therefore the first large larval masses were in the head. The temperatures recorded in the head region, both by the internal probe and the infrared thermometer showed a steady increase in temperature resulting in an elevated temperature above ambient, especially during the night. The most striking aspect of the larval mass temperatures is the break with the ambient diurnal cycle i.e. the temperatures rise at a constant rate until they reach their maximum value (Cianci & Sheldon, 1990). The greatest difference in temperature was recorded on the morning of the fifth day, with a 20ºC increase above the ambient temperature. The internal temperatures continued to rise above ambient, up to a maximum of 45ºC, above which the larvae would be unable to survive. This may also be the reason for the observed churning movement of the larvae, where they move in and out of the centre of the larval mass, heating up in the core and cooling down on the periphery. Having reached the highest temperature, the internal probes recorded decreased temperatures which began to fluctuate more with the ambient temperature, although still significantly raised above it by approximately 15-17ºC. This may have been due to the buffering effects of being inside the body, thus the temperature remained elevated above the ambient, even when no larval mass was apparent.

In contrast, there was a considerable delay before any flies were able to get access to the body in the bin, more so than with indoor bodies (Reibe & Madea, 2010) and the blowflies were not the first to gain access, but after seven days *Megaselia scalaris* (Phoridae) had accessed the bin. They are known to develop in vegetation, carrion or excrement, and Motter (1898) reported Phorids being attracted in great numbers to a box in which a dog cadaver had been interred, and which was emitting a "very pungent ammoniacal odor". Campobasso *et al*. (2004) reported *Megaselia scalaris* on an exhumed body in Southern Italy, and *Megaselia abdita* has been reported in a number of forensic cases (Disney & Manlove, 2005; Manlove & Disney, 2007), suggesting that although usually relegated to a secondary forensic role, when larger flies are prevented from colonizing a body due to reduced access, such as burial, Phorid species may occur in the early stages of decomposition and therefore give a more accurate minPMI estimate. In this study, they appeared to be feeding on the fluids seeping out of the body, and were found crawling around the inner surfaces of the bin and lid, and on the surface of the body. Large quantities of puparia were found in rafts on the outer rim of the bin under the lid. The internal body temperatures were still elevated after four days, even though no large Calliphorid masses had been established at this time, possibly due to bacterial activity inside the body, which has previously been recorded in buried bodies (Rodriguez & Bass, 1985). Due to the large amount of fluid accumulating at the inside bottom of the bin, and the high levels of ammonia, the Calliphorid larvae were unable to pupate inside the bin, so moved outside to find a pupation site, as the inside of the bin was not a suitable pupation area. Phorid larvae were able to attach to the side of the bin in order to pupate, but this was not possible for the Calliphorids, which dropped into the surrounding soil.

Thermal imaging was used to monitor larval mass temperatures on a body at the ARF. Elevated larval temperatures of 45°C were recorded, 15°C above the ambient temperature. As well as the benefit of being non-invasive and being able to view the temperatures of an entire body, thermal imaging also gave the benefit of showing where the hottest larval masses were, which was not necessarily where the larvae were most visible. With thermal imaging, it was also possible to show the retention of heat of larval masses, and the movement

of individual larvae into and out of the mass, where they rapidly lose heat on the periphery of the mass. If, however, a larval mass was uncovered, the larvae quickly dispersed and the local temperature dropped rapidly. Slone & Gruner (2007) noted that groups of loosely associated larvae had lower temperatures than those in tight aggregations, which explains why the heat dissipated quickly when the larvae were disturbed. Thermal imaging also demonstrated that larger larval masses have a higher temperature than smaller larval masses, although the relationship is not linear. Even small larval masses may hold heat of over 40°C, and none can be over 45°C, regardless of size, otherwise the larvae would overheat and die. Slone and Gruner (2007) suggested that smaller larval masses were influenced by ambient air temperature, but that larger larval masses, those greater than 20cm^3 had strongly regulated internal temperatures, determined only by the volume of the mass.

Observations of the dispersal behaviour of post-feeding Calliphorid larvae demonstrated that the number of days, distance covered, direction followed, and the pattern of dispersal may vary considerably. Tessmer & Meek (1996) suggested that larvae move off a body mainly in a southerly direction. In this study, however, although larvae dispersed in this direction from three bodies in this study, larvae from the other three bodies dispersed in a northerly direction. Where a body was lying on an obvious slope, the larvae moved downhill, partly because that was the direction in which the body fluids were flowing onto the ground and also because it required less effort for the larvae. In some cases, however, larvae were observed to be moving uphill. Dispersing larvae sometimes followed a narrow trail (Arnott & Turner, 2008), and depending on soil type and amount of surrounding vegetation, it may be possible to see such trails, and therefore assess whether larvae have already started leaving the body, and if the trail is visible, to find the pupariating larvae. In some cases, however, movement off a body appears to be random (Gomes & Von Zuben, 2005), such as when the larvae radiated out in all directions from the body. Field studies by Kočárek (2001) suggested that larvae disperse from a body at night, in order to avoid predation, a behaviour which observations in Tennessee would support, as larvae were seen dispersing from bodies early in the morning on a number of occasions.

CHAPTER 5

PIG VERSUS HUMAN DECOMPOSITION

5.1 Introduction

Forensic entomology studies have been carried out by researchers on a wide variety of different animal species, but the most widely used animal model is the domestic pig (*Sus scrofa domestica*) (Goff, 2000), for three main reasons: economical, ethical and biological. Firstly, pigs are bred for consumption and are therefore easy and cheap to acquire, especially compared to non-human primates. Secondly, because they are consumed by humans, rather than considered as domestic pets, their use in research is more acceptable to the public, students and their research institutions. Thirdly, although humans are closer to other primates in their genetic make-up, pigs are a good model for humans in many aspects of infant and adult anatomy, physiology, biochemistry, pathology, pharmacology and skin type. Studies have shown that the cadaveric volatile organic compounds (VOCs) produced during decomposition are similar between pig and human cadavers (Dekeirsschieter *et al*, 2009).

Despite the suggestion that the use of human cadavers for the study of Calliphorid development is not practical for large numbers of replicates (Slone & Gruner, 200&), Rodriguez & Bass (1983) conducted studies on the insect activity associated with four human cadavers at different times of the year at the Anthropological Research Facility in Knoxville, Tennessee. They reported that the succession of insect families and species was consistent with those described by Reed (1958) in his study of 43 dog carcasses carried out over a year long period in an area about one mile south of the city limits of Knoxville. Although they reported variations in the minimum colonisation time, which they attributed to the different rates of decomposition between humans and dogs due to differences in body size and composition, they did not give further information about which family of insect they were referring to. Also, Reed (1958) made less frequent visits to the carcasses, only once in 12 days during the coldest

period, and therefore the first period of colonisation of some species may not have been observed and recorded.

Although animals, and specifically pigs, are generally accepted as a suitable model for humans in forensic entomology studies, it is likely that in the future, courts may question the validity of scientific research carried out on non-human rather than human cadavers (Catts & Goff, 1992; Goff, 1993). A study carried out in 1989 at the Anthropological Research Facility in Tennessee endeavoured to validate the use of the domestic pig (*Sus scrofa* L.) as a model for humans in forensic entomology studies using two pig cadavers and one human cadaver (Schoenly *et al*., 2007). The composition and succession of insects was found to be comparable between the two types of cadaver with three quarters of the forensically important taxa (47/64) collected on all three subjects, although without replicates of the human cadaver the data is of limited validity. Schoenly *et al*., (2007) stated, however, that the arthropod faunas overlapped sufficiently to recommend substituting pigs for humans in forensic entomology studies. They also stressed, however, that as no data were collected regarding Calliphorid larval development, no conclusions could be drawn as to whether pigs are a suitable model for humans in estimating minimum PMI.

5.2 Aims

The aims were to compare pig and human cadavers with regards to the rate of decomposition; the rate and sites of oviposition by blowflies; the composition and succession of insects; the development rate of blowfly larvae; formation and location of larval masses and the associated elevated temperatures. The hypothesis was that there would be no significant difference between pigs and humans, thus validating the use of pigs as a model for humans in forensic entomology studies.

5.3 Materials and methods

5.3.1 Study site

The site used was the Anthropological Research Facility, Knoxville, Tennessee, USA, details of which are outlined in Section 4.1.

5.3.2 Experimental models

A total of six pig cadavers and six adult human males were used; three pigs and three humans in two successive years: July 2008 (Experiment 1) and July 2009 (Experiment 2). Details of the human donor programme are outlined in Section 4.1. The pigs were three to four months old, each weighing approximately 45 kilos, and were purchased from a local pig farm and killed with a single shot to the head, in compliance with HMSA (The Humane Methods of Slaughter Act) regulations. This weight of pigs was chosen so that the torso was of a comparable size to that of the human cadavers. In the first experiment the pigs were killed nine days prior to the start of the experiment, during which time they were stored in a chest freezer; in the second experiment the pigs were killed the day before the start of the experiment, and stored overnight in a chest freezer. The six human cadavers were male and were stored in body bags in a chest freezer until the start of each experiment. Details of age, weight and length of time stored frozen are shown in Table 5.3.2.1.

Table 5.3.2.1 Age (years) and weight (kilograms) of six male human cadavers used in the study, and number of days each was held in frozen storage before 48 hours of defrosting prior to the start of experiment.

Exp.	Cadaver	Age (yrs)	Weight (kg)	Frozen storage (days)
1	Man 1A	65	91	31
	Man 1B	54	73	33
	Man 1C	59	68	16
2	Man 2A	55	64	24
	Man 2B	57	89	25
	Man 2C	76	118	25

5.3.3 Placement of cadavers

All cadavers were removed from the chest freezer 48 hours before the start of the experiment and laid on the ground outside to defrost. During this time, the pigs were tightly wrapped in large plastic bags and the human cadavers were in sealed body bags. The wrappings were carefully checked to ensure that no insects could gain access to the bodies during the defrosting process, and the bodies were checked prior to the start of the experiments to ensure that there were no blowfly eggs already present on them.

The bodies were laid naked on the ground. Each paired pig and human were placed five to six metres apart to reduce differences caused by location. Each pig-human pair was placed at least 50m apart to ensure each pair was independent of the other pairs. Each cadaver had two Tinytag® Plus datalogger probes inserted into the mouth and rectum, which had previously been set to record the temperature every 15 minutes. In Experiment 1, a Tinytag® Plus ambient datalogger was placed half way between each pig-human pair. In Experiment 2, a single Tinytag® Plus ambient datalogger was placed approximately equidistant from all three pig-human pairs.

5.3.4 Sampling procedure

Twice a day, at approximately 08:30 and 15:30, each cadaver was visited. At each visit, a TN1 non-contact infrared thermometer was used to record surface

body temperatures and the surface temperature of any conspicuous Calliphorid larval masses. Larval samples of at least 100 individuals were also collected at each visit, killed in freshly boiled water, and then preserved in 70% ethanol.

During Experiment 1, sticky traps were used to collect mainly airborne insects from each cadaver simultaneously, as they are an inexpensive and efficient way of collecting large samples of blowflies (Schoenly *et al.*, 2007) and are more efficient at collecting larger numbers of individuals than some other fly traps (Hall *et al.*, 2003). The sticky traps measured 15cm x 20cm and were placed by the head of each body 24 hours after the start of the experiment. They were left for 24 hours before being removed and stored in a freezer. New sticky traps were put out at 48 hour intervals, therefore there was a continuum of 24 hours with sticky trap, followed by 24 hours without for the duration of the experiment. In total there were seven sticky traps per body put out over a 15 day period. A control sticky trap was also put during the same periods, approximately 20m from the nearest Man-Pig pair.

In addition, thermal imaging was carried out during Experiment 1 using a FLIR Systems ThermaCAM® P640 camera. Images were taken of all three pig and human bodies between Days 2 and 10 at the following times: Day 2 (pm only), Days 3, 4, 7, 8 & 10 (am and pm), Days 6 & 9 (am only), Day 5 (am, pm and eve).

5.3.5 Laboratory procedure

Sticky traps were rolled up and stored in a freezer, before being transported back to the UK by courier. All wet samples were sealed in their tubes using Parafilm®M, before being sent back to the UK by courier. All samples were examined under a binocular microscope using up to x50 magnification. Larvae were measured using an eyepiece graticule. Specimens were identified using keys from Smith (1986), Greenberg & Kunich (2002) and Szpila (2010).

5.4 Results

5.4.1 Photographs

Figure 5.4.1.1 Three human and three pig cadavers used in Experiment 1, carried out in July 2008, shown on the afternoon of Days 1, 7 and 15

Figure 5.4.1.2 Three human and three pig cadavers used in Experiment 2, carried out in July 2009, shown on the afternoon of Days 1, 8 and 16

The photographs in Figures 5.4.1.1 (Experiment 1) and 5.4.1.2 (Experiment 2) show a significant amount of decomposition by the end of the experiment in all twelve cadavers, although there was some variation.

In general, the pig cadavers appeared to undergo a greater degree of decomposition than the humans (although post-experiment weighing was not carried out), and by the end of each experiment, all twelve pig cadavers were highly decomposed and mummified with at least part of the skeleton visible. Pigs 2B and 2C, especially, already showed a large amount of soft tissue removal by Day 8 of the experiment. All three pigs in Experiment 1 and also Pig 2A had large larval masses on them by Day 7/8, i.e. midway through the experiment, but still a significant amount of fresh tissue remaining by the end of the experiment, Day 15/16.

All of the six human cadavers showed significant bloating and larval masses by Day 7/8, i.e. midway, of the experiments. By the end of the experiments, four of the six cadavers had reached a similar stage of decomposition, becoming partially mummified, although not completely dry. Two of the six human cadavers, however, differed from the other four. Man 1A became more bloated during the active decay stage, and by the end of the experiment, Day 15, still had feeding larval masses present on it, while the other five cadavers in the same experiment were de-bloated and partially mummified by this stage. Man 2C showed a greater degree of decomposition with extensive skeletonisation by the end of the experiment (Day 16), than all the other human cadavers, and its stage of decomposition more closely resembled that of the pig cadavers than the other five human cadavers.

5.4.2 Rate of decomposition

Key to decomposition stages				
Fresh	Bloat	Active decay	Advanced decay	Dry/Skeletal

Table 5.4.2.1 Observations of decomposition and immature development stages for Man 1A

Day of Expt.	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Decomp															
Egg masses															
1st instar															
2nd instar															
3rd instar															
Post-feeding															

Table 5.4.2.2 Observations of decomposition and immature development stages for Pig 1A

Day of Expt.	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Decomp															
Egg masses															
1st instar															
2nd instar															
3rd instar															
Post-feeding															

Table 5.4.2.3 Observations of decomposition and immature development stages for Man 1B

Day of Expt.	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Decomp															
Egg masses															
1st instar															
2nd instar															
3rd instar															
Post-feeding															

Table 5.4.2.4 Observations of decomposition and immature development stages for Pig 1B

Day of Expt.	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Decomp															
Egg masses															
1st instar															
2nd instar															
3rd instar															
Post-feeding															

Table 5.4.2.5 Observations of decomposition and immature development stages for Man 1C

Day of Expt.	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Decomp															
Egg masses															
1st instar															
2nd instar															
3rd instar															
Post-feeding															

Table 5.4.2.6 Observations of decomposition and immature development stages for Pig 1C

Day of Expt.	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Decomp															
Egg masses															
1st instar															
2nd instar															
3rd instar															
Post-feeding															

Table 5.4.2.7 Observations of decomposition and immature development stages for Man 2A

Day of Expt.	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
Decomp																
Egg masses																
1st instar																
2nd instar																
3rd instar																
Post-feeding																

Table 5.4.2.8 Observations of decomposition and immature development stages for Pig 2A

Day of Expt.	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
Decomp																
Egg masses																
1st instar																
2nd instar																
3rd instar																
Post-feeding																

Table 5.4.2.9 Observations of decomposition and immature development stages for Man 2B

Day of Expt.	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
Decomp																
Egg masses																
1st instar																
2nd instar																
3rd instar																
Post-feeding																

Table 5.4.2.10 Observations of decomposition and immature development stages for Pig 2B

Day of Expt.	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
Decomp																
Egg masses																
1st instar																
2nd instar																
3rd instar																
Post-feeding																

Table 5.4.2.11 Observations of decomposition and immature development stages for Man 2C

Day of Expt.	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
Decomp																
Egg masses																
1st instar																
2nd instar																
3rd instar																
Post-feeding																

Table 5.4.2.12 Observations of decomposition and immature development stages for Pig 2C

Day of Expt.	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
Decomp																
Egg masses																
1st instar																
2nd instar																
3rd instar																
Post-feeding																

Although there is a recognised order of decomposition stages, as outlined in the description of the five stages referred to in this study, not all the cadavers followed this order. Man 1A was mildly bloated on Days 6 and 7, but having gone into the active decay stage, i.e. with larval masses feeding on it, became bloated again, which can be seen in the photograph taken on Day 15 (Figure 5.4.1.1). Pig 2B and Man 2C went directly from the active decay stage into the dry/skeletal stage, without really going through the advanced stage of decomposition. Although appearing to be "fresh", Man 2B was already fairly bloated when he was laid out on Day 1 and had extensive marbling; the cadaver did not become any more bloated during the experiment.

All but one of the cadavers had blowflies landing on them within minutes of being laid out; flies were not observed on Man 2C until Day 2. All cadavers, except Man 2C, had egg masses on them by the end of Day 1 and 1st instars present from Day 2. Man 2C had the first eggs laid on the body on Day 2, and 1st instars were observed on Day 3. In Experiment 1, there was also a second distinct wave of egg-laying on Man-Pig pairs 1A and 1B, but not on Man-Pig pair 1C. In Experiment 2, there was no further egg-laying after Day 6. 2nd and 3rd instar stages followed a day or two after 1st instars in all cadavers, and post-feeding larvae were observed between three and nine days later. In Experiment 1, 1st, 2nd and 3rd instars were observed on Man-Pig pairs 1A and 1B throughout the experiment, whereas in Man-Pig pair 1C 1st and 2nd instars were only observed in the first half of the experiment. In Experiment 2, 1st instars were only observed in the first half of the experiment in Man-Pig pairs 2B and 2C, but were present in Man-Pig pair 2A for longer.

In Experiment 1, post-feeding larvae were observed from Day 8 in Man-Pig pairs 1B and 1C, and from Day 9 in Man-Pig pair 1A, and continued until the end of the experiment for all cadavers. In Experiment 2, post-feeding larvae were observed starting between Days 10 and 13. Dispersal from the body continued to the end of the experiment in all three men and Pig 2A, but was not observed after Day 12 in Pig 2B and Day 13 in Pig 2C.

The data in Tables 5.4.2.1 to 5.4.2.12 were combined to show the insect development on the bodies for all six men (Table 5.4.2.13) and all six pigs (Table 5.4.2.14).

1 2 3 4 5 6 Number of men/pigs

Table 5.4.2.13 Combined data for men (n=6) over both years

Men (n=6)	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Egg masses															
1st instar															
2nd instar															
3rd instar															
Post-feeding															

Table 5.4.2.14 Combined data for pigs (n=6) over both years

Pigs (n=6)	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Egg masses															
1st instar															
2nd instar															
3rd instar															
Post-feeding															

The data in Tables 5.4.2.1 to 5.4.2.12 were also combined to show to mean day of first observation of each stage of blowfly development for the 12 cadavers.

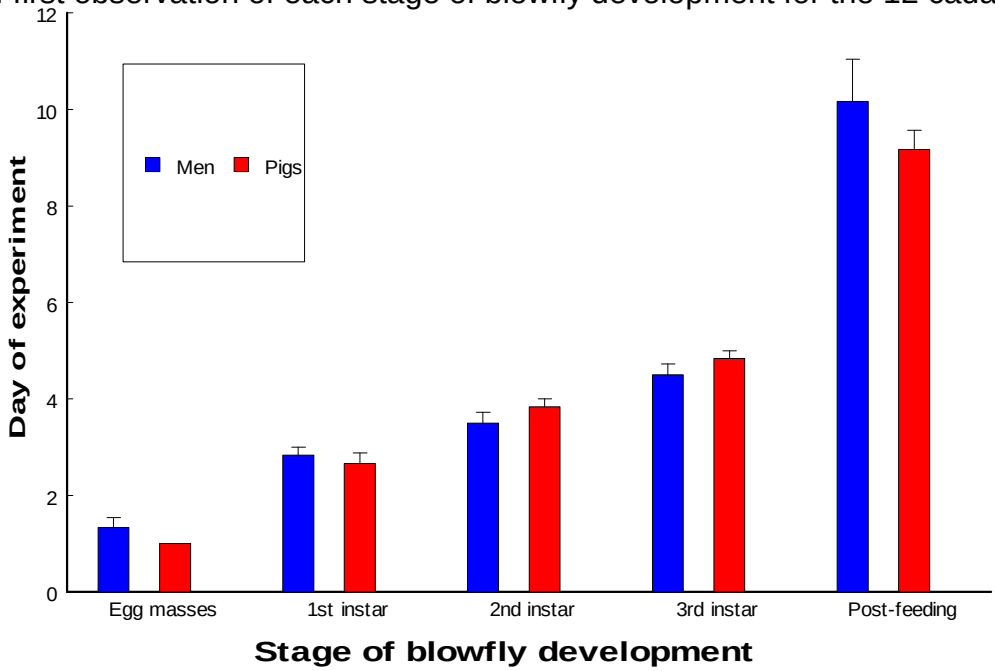


Figure 5.4.2.1 Mean day of first observation of each stage blowfly of development from six pig (red) and six human (blue) cadavers.

There was a very similar pattern of oviposition on both the pigs and men, with eggs being laid mainly in the body orifices, and mostly in head region. Oviposition, however, appears to continue for longer on the pigs than on the humans: oviposition was observed on 14 out of 15 days on the pigs and on 10 out of 15 days on the men. The 1st, 2nd and 3rd instar stages were observed for a comparable amount of time on both types of cadaver. The post-feeding stage commences on the same day (Day 8) on both men and pigs although the majority of larvae disperse away from the pigs earlier than from the men. In both the pigs and the men, there is some variation, with all larval stages being observed on one cadaver of each type one day earlier than on the other five cadavers.

5.4.3 Sticky traps (Experiment 1)

5.4.3.1 Raw data

The numbers of individuals of seven species of blowfly captured on sticky traps are shown in Appendix I, Table 5.4.3.1.

5.4.3.2 Photographs

The main Order of insects collected were Diptera, which included the following Families and Species: Calliphoridae (*Lucilia illustris*, *L. sericata, L. coeruleiviridis, Phormia regina, Cochliomyia macellaria and Chrysomya rufifaces*; Stratiomyidae *(Hermetia illucens),* Sarcophagidae, Muscidae, Fannidae, Sepsidae, Piophilidae and Phoridae. The following Coleoptera were also collected: Histeridae, Staphylinidae, Dermestidae, Cleridae and Trogidae. Also some species of Hymenoptera, Vespidae and Formicidae were collected.

The sticky traps from Experiment 1 are shown in Appendix 1, Figures 5.4.3.1 to 5.4.3.7. The Control sticky traps attracted far fewer individuals in general than the Pig and Man sticky traps, and those insects that were trapped were mainly wasps. The greatest number of individuals were trapped in the first three series

of traps (i.e Day 2-3, 4-5 and 6-7), with Man 1B, Pig 1B and Pig 1C attracting a greater number of individuals than Man 1A, Pig 1A and Man 1C.

5.4.3.3 Data

The graphs shown in this section present the results for the most dominant species of insect, the Calliphoridae and Stratiomyidae. The results for the Control sticky traps and the Key of Species for all graphs are shown in Figure 5.4.3.8 below. The numbers of individuals caught on the Control sticky trap were very low (n=19, 0.8% of total catch), with no flies being trapped on Days 4-5, 8-9 and 14-15. The most numerous and regularly trapped on the Control sticky traps were *Cochliomyia macellaria* (47.4%), with *Lucilia illustris* (15.8%) also being trapped on Days 2-3 and 6-7, *Phormia regina* (26.3%) on Day 10-11 and *Lucilia sericata* (10.5%) on Days 10-11 and 12-13.

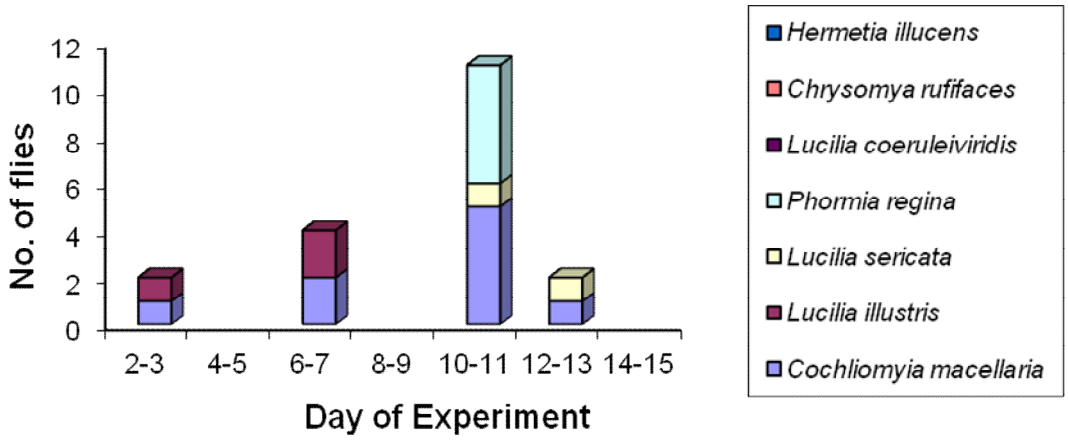


Figure 5.4.3.8 Control sticky traps and key of species (Calliphoridae and Stratiomyidae) for all graphs in Section 5.4.3.2.

Figure 5.4.3.9 shows the species numbers collected for each individual cadaver. Days 2-3, 4-5 and 6-7 had the greatest numbers of individual flies trapped on all six cadavers. The greatest numbers of flies trapped on Day 2-3 were on Man 1B, Pig 1B and Pig 1C, the dominant species being *Lucilia coeruleiviridis*. The second most dominant species on Man 1B and Pig 1B was *Lucilia illustris*, followed by *Phormia regina*. Man 1A and Pig 1A trapped a larger number of flies on Day 4-5 than on Day 2-3, as did Pig 1B by a small number. From the fourth day of trapping, Day 8-9, the number of flies caught decreased to fewer than 50 individuals per cadaver, with *Hermetia illucens* and *Cochliomyia macellaria* being the most dominant species.

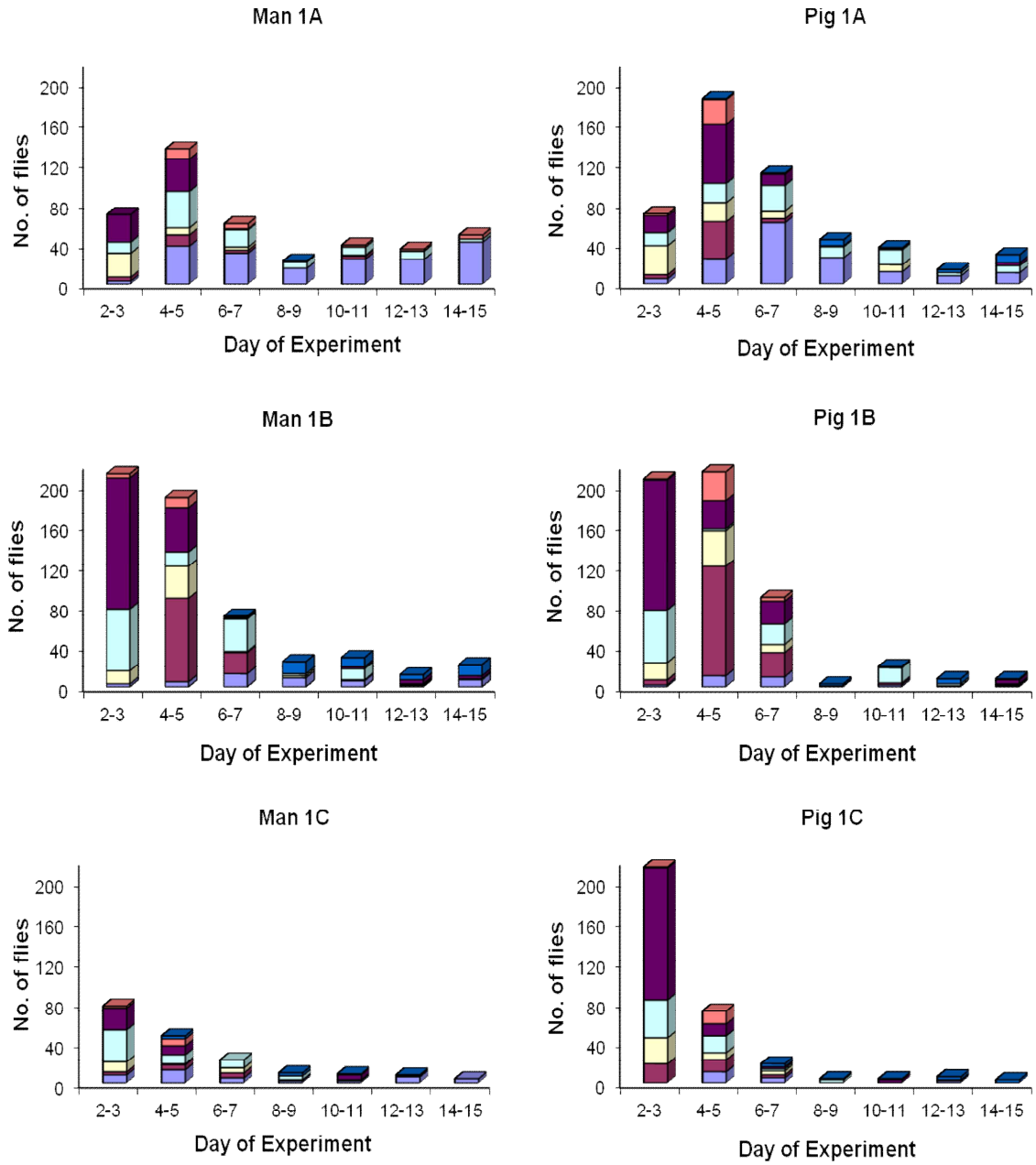


Figure 5.4.3.9 Numbers of individuals of seven Calliphoridae species caught on sticky traps for individual cadavers (Experiment 1)

The data for each of the three Man-Pig pairs are shown in Figure 5.4.3.10. As suggested by the graphs for individual cadavers, the greatest number of flies was trapped on Man-Pig pair 1B on Days 2-3 and 4-5. The number reduced by more than 50% on Day 6-7 and from Day 8-9 onwards the numbers were very low. The species composition on the first two trapping days for Man-Pig pair 1B was very different, with *Lucilia coeruleiviridis* being dominant on Day 2-3 and *Lucilia illustris* being dominant on Day 4-5. Man-Pig pair 1A trapped more flies on Day 4-5 than on Day 2-3, with the species composition not being dominated by any one species. Although Man-Pig pair 1C trapped fewer individuals on

Day 2-3 than Man-Pig pair 1B, the species composition for these two Man-Pig pairs was very similar.

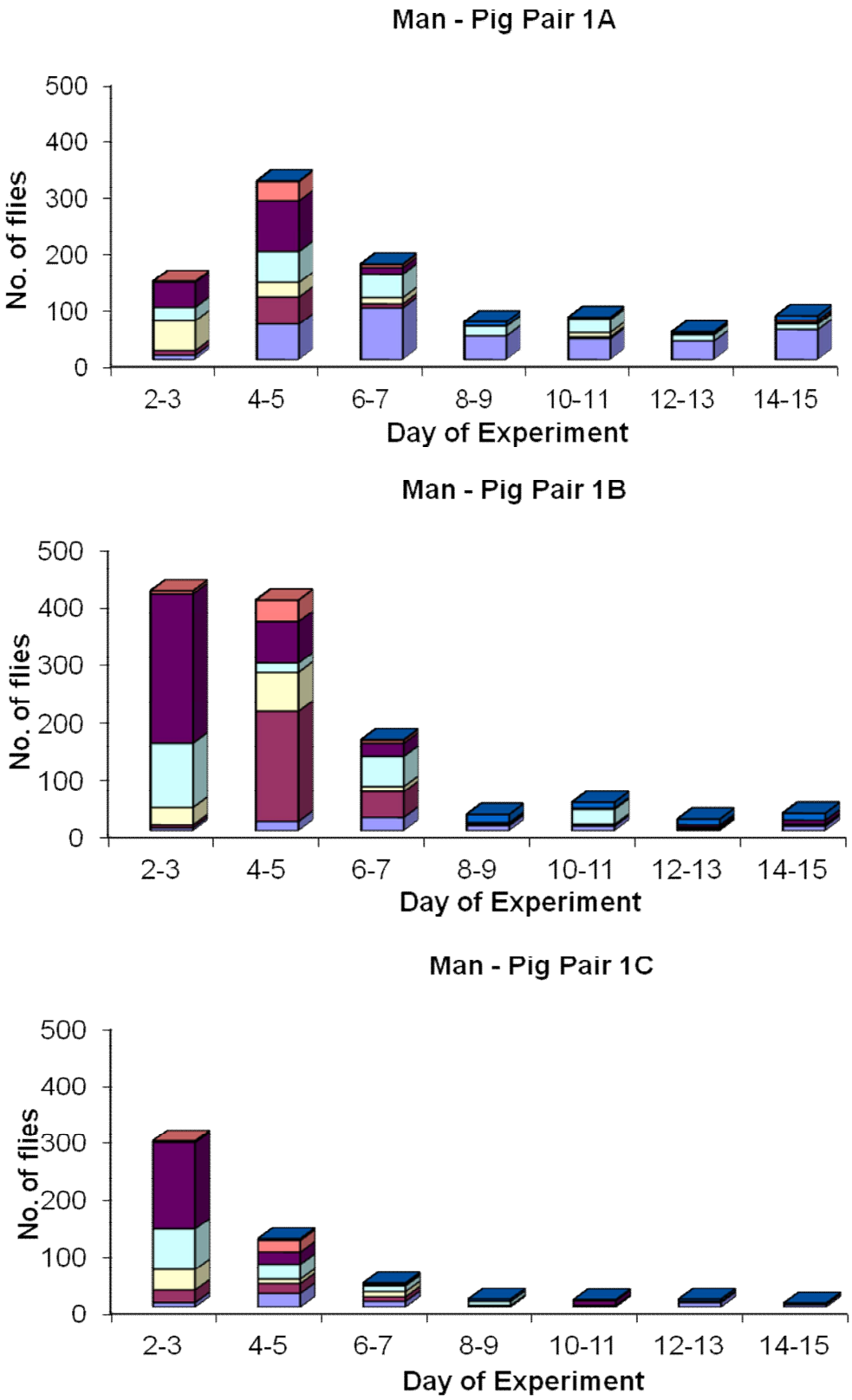


Figure 5.4.3.10 Numbers of individuals of seven Calliphoridae species caught on sticky traps for three Man-Pig pairs (Experiment 1)

The combined data for Men and Pigs is shown in Figure 5.4.3.11. Overall the pig cadavers attracted greater numbers of flies than the male cadavers: total catch was 2519 individuals, the men attracted 45.4%, the pigs 53.8%, and the Control 0.8%. The species composition between the two types of cadaver was, however, very similar. *Lucilia coeruleviridis* was the dominant species on Day 2-3, with *Phormia regina* being the second most dominant species. The composition of fly species was more varied on Day 4-5 for both types of cadaver, with *Lucilia illustris* becoming proportionally more abundant. The number of flies was reduced by about 50% on Day 6-7 for both Men and Pigs, and from Day 8-9 onwards the numbers were reduced yet further.

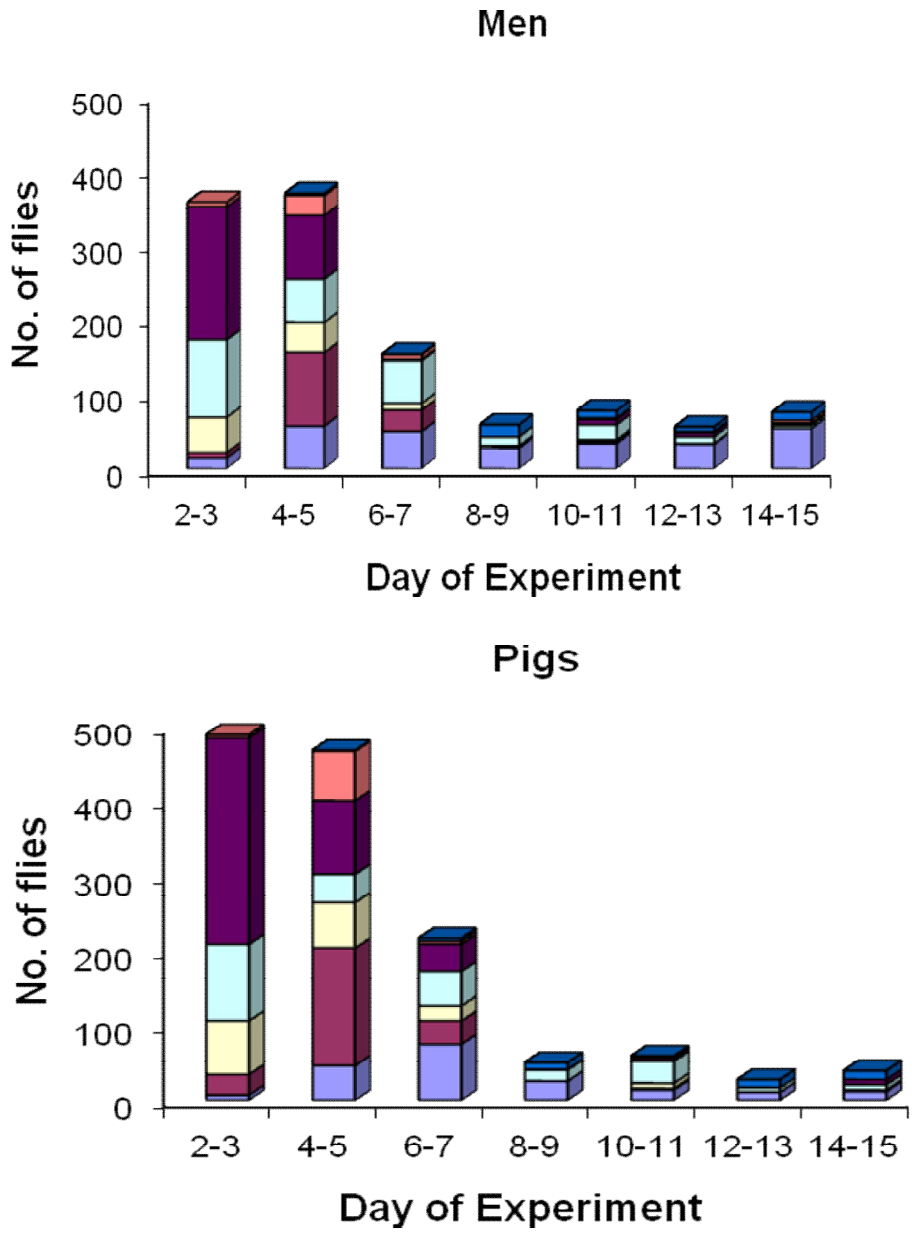


Figure 5.4.3.11 Numbers of individuals of seven Calliphoridae species caught on sticky traps for three Male and three Pig cadavers (Experiment 1)

5.4.3.4 Percentage similarity

Based on the total numbers of individuals of each fly species caught on the sticky traps, the percentage similarity between groups was calculated i.e. the sum of the lowest percent value of a species between groups.

Table 5.4.3.2 Percentage similarity between A) the Man and Pig in each pair; B) the 3 Men; C) the 3 Pigs; D) the 3 Man-Pig pairs; E) the 3 Men and 3 Pigs

	Comparison	**Percentage**	**Mean (n=3)**
A	Man 1A to Pig 1A	83.6%	80.1%
	Man 1B to Pig 1B	86.8%	
	Man 1C to Pig 1C	69.9%	
B	Man 1A to Man 1B	61.0%	71.0%
	Man 1A to Man 1C	77.9%	
	Man 1B to Man 1C	74.1%	
C	Pig 1A to Pig 1B	68.0%	74.2%
	Pig 1A to Pig 1C	71.6%	
	Pig 1B to Pig 1C	83.1%	
D	Pair 1A to Pair 1B	66.8%	75.8%
	Pair 1A to Pair 1C	73.9%	
	Pair 1B to Pair 1C	86.7%	
E	3 Men to 3 Pigs	85.0%	

Within each Man-Pig pair, the lowest similarity was 69.9% in pair 1C, whereas it was relatively high within pair 1A (83.6%) and pair 1B (86.8%). The lowest similarity in species composition occurred between Man-Pig pair 1A and Man-Pig pair 1B, where there was only 61.0% similarity between Man 1A and Man 1B, a 68.0% similarity between Pig 1A and Pig 1B, and 66.8% between the two Man-Pig pairs. In contrast, there was a relatively high similarity between pairs 1B and 1C, with 83.1% similarity between Pig 1B and Pig 1C and 86.7% similarity between Man-Pig pairs 1B and 1C. Overall, there was an 85.0% similarity in species composition between the combined data for the 3 Men and the 3 Pigs.

5.4.3.5 Principal Components Analysis

Based on the total numbers of individuals of each fly species caught on the sticky traps, the differences between the six cadavers was investigated using Principal Components Analysis, shown in Figure 5.4.3.12.

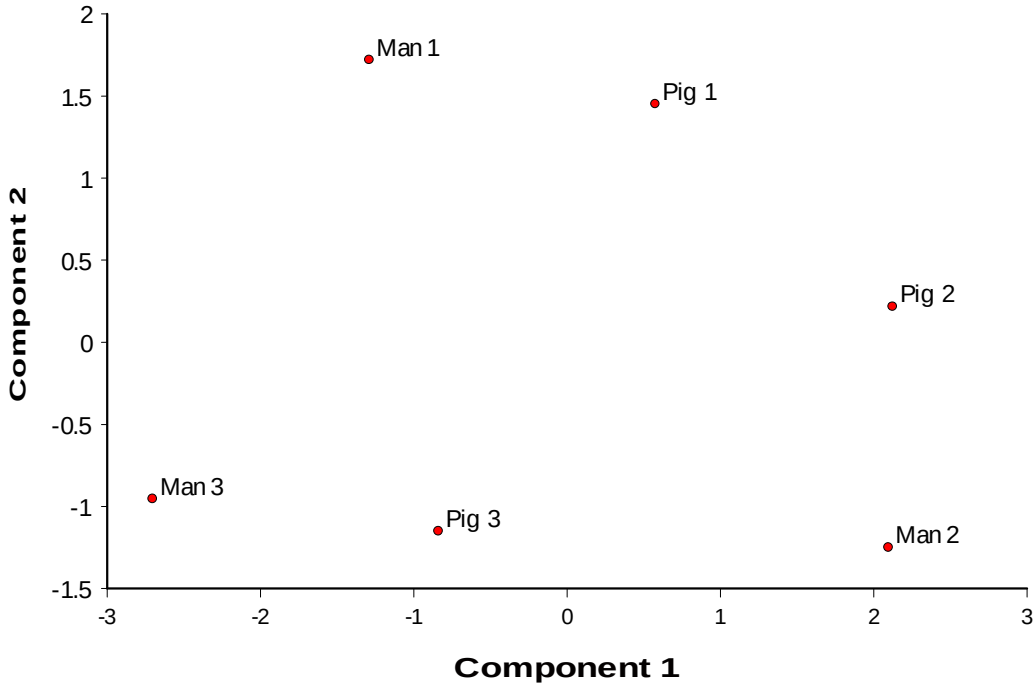


Figure 5.4.3.12 PCA plot with two components of seven blowfly species caught on sticky traps placed near six cadavers (Man 1-3 and Pig 1-3) (Experiment 1, July 2008)

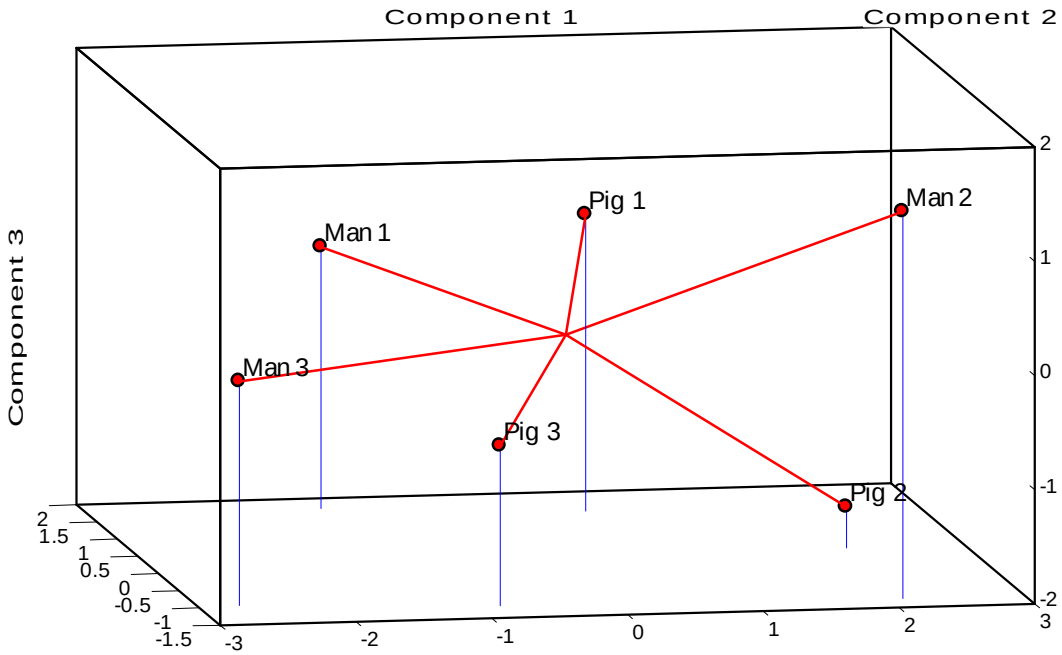


Figure 5.4.3.13 PCA plot with three components of seven blowfly species caught on sticky traps placed near six cadavers (Man 1-3 and Pig 1-3) (Experiment 1, July 2008)

5.4.4 Larval development (Experiment 1)

Figure 5.4.4.1 shows the mean larval length of *Phormia regina* larvae collected from the mouth and genitals of each cadaver in Experiment 1 (each sample n=20). The graph indicates that the larvae collected from the mouths of the men and pigs developed first. The larvae collected from the genitals of both the men and pigs were approximately one day behind the larvae collected from the mouths of both pigs and men, but the from Day 6 all four samples were within 2mm difference. All larvae developed to a maximum length of between 12.5mm and 14mm.

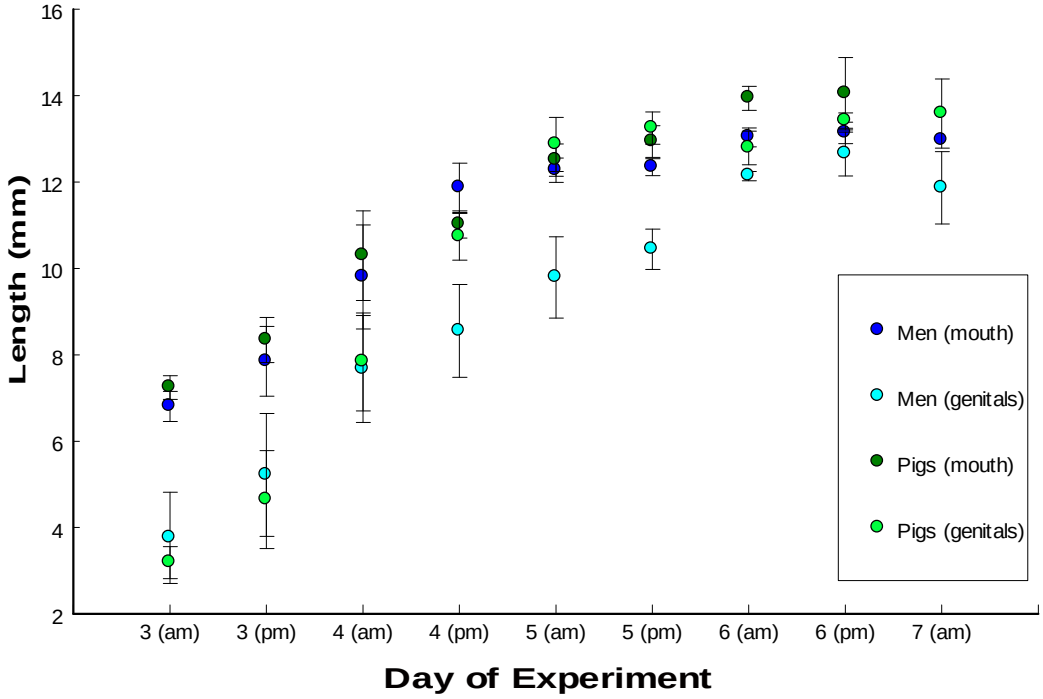


Figure 5.4.4.1 Plot of mean ±se larval lengths of *Phormia regina* from Experiment 1, July 2008, from six cadavers (three men and 3 pigs) (Each sample n=20)

5.4.5 Dataloggers

5.4.5.1 Ambient temperatures

The experiments were carried out over two successive years, in July 2008 and July 2009. Therefore the ambient temperatures in the two years were plotted (Figure 5.4.5.1) to ascertain whether there was any significant difference in temperature between the two years.

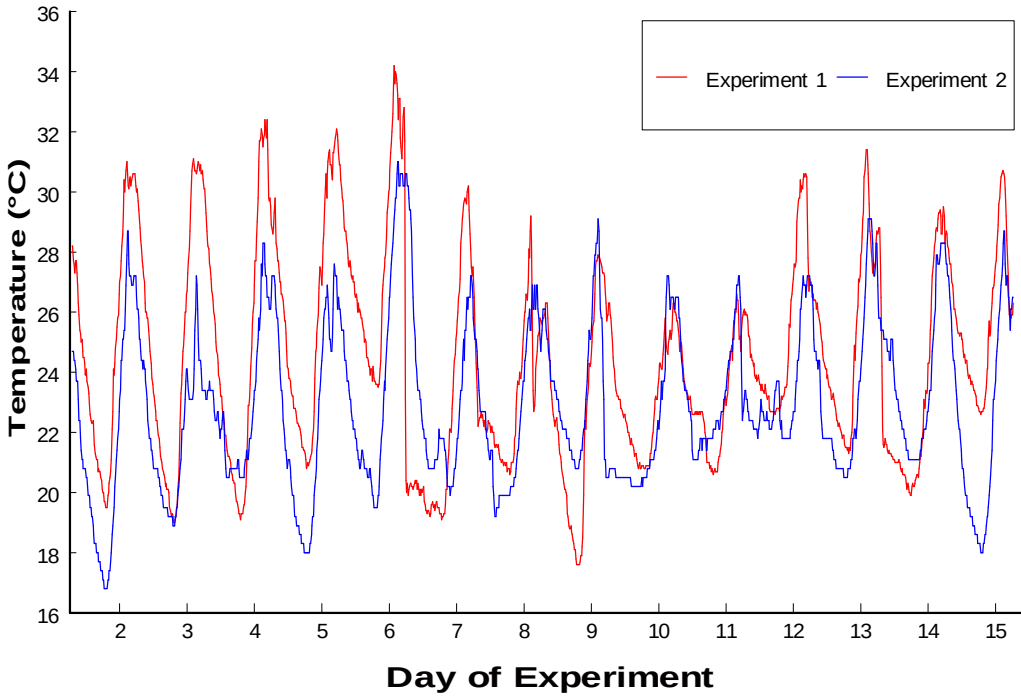


Figure 5.4.5.1 Ambient temperatures during Experiment 1 in July 2008 (red) and Experiment 2 in July 2009 (blue) from Day 1 to 15. The Day of Experiment is marked at 12:00 midday.

The temperatures ranged from 17.6°C to 34.2°C (range 16.6°C) during Experiment 1, and 16.8°C to 31.0°C (range 14.2°C) during Experiment 2. The mean temperature was 24.5°C in Experiment 1 and 22.9°C in Experiment 2, a difference of 1.6°C. Therefore the temperatures were only slightly higher overall in Experiment 1 than in Experiment 2.

5.4.5.2 Basic statistics

Basic statistics for all the internal dataloggers used in the cadavers are given in Tables 5.4.5.1 (Experiment 1) and 5.4.5.2 (Experiment 2). The minimum rectal temperatures were low for all cadavers with some of them starting below 0°C. This was due to the body core temperatures taking 2-3 days to reach ambient (Figures 5.4.5.3 to 5.4.5.8), having been stored in freezers prior to the start of the experiments.

Table 5.4.5.1 Experiment 1 datalogger statistics from 19:00 on 16/07/08 (Day 1) to 18:30 on 30/07/09 (Day 15)

Datalogger	Min (°C)	Max (°C)	Range (°C)	Mean (°C)	SD
1A ambient	17.4	35.2	17.8	25.0	±3.7
Man 1A (mouth)	17.1	39.9	22.8	27.9	±3.5
Man 1A (rectum)	-2.5	36.6	39.1	25.1	±7.6
Pig 1A (rectum)	-1.4	42.0	43.4	29.1	±7.3
1B ambient	17.4	34.8	17.4	24.3	±3.5
Man 1B (mouth)	7.5	46.0	38.5	32.6	±5.7
Man 1B (rectum)	-2.7	41.5	44.2	27.0	±9.0
Pig 1B (mouth)	14.5	42.6	28.1	29.6	±5.6
Pig 1B (rectum)	5.1	34.8	29.7	26.5	±4.4
1C ambient	17.7	33.5	15.8	24.2	±3.4
Man 1C (mouth)	7.7	46.6	38.9	32.0	±6.8
Man 1C (rectum)	-2.7	47.2	49.9	29.4	±10.4
Pig 1C (mouth)	14.0	43.1	29.1	32.3	±6.0
Pig 1C (rectum)	-0.4	37.5	37.9	26.9	±5.9

Table 5.4.5.2 Experiment 2 datalogger statistics from 19:00 on 20/07/09 (Day 1) to 10:00 on 04/08/09 (Day 16)

Datalogger	Min (°C)	Max (°C)	Range (°C)	Mean (°C)	SD
Ambient	16.8	31.0	14.2	22.8	±2.8
Man 2A (mouth)	17.7	42.0	24.3	27.6	±5.4
Man 2A (rectum)	-0.9	37.5	38.4	26.2	±6.6
Pig 2A (mouth)	8.8	46.6	37.8	29.0	±7.7
Pig 2A (rectum)	3.4	39.4	36.0	25.8	±5.6
Man 2B (mouth)	17.7	44.2	26.5	29.7	±6.0
Man 2B (rectum)	6.4	32.2	25.8	25.5	±4.9
Pig 2B (mouth)	8.3	41.5	33.2	26.9	±7.0
Pig 2B (rectum)	16.8	39.9	23.1	25.6	±5.0
Man 2C (mouth)	18.3	46.6	28.3	32.0	±5.3
Man 2C (rectum)	2.8	46.6	43.8	31.0	±6.9
Pig 2C (mouth)	18.9	51.9	33.0	29.2	±7.1
Pig 2C (rectum)	12.6	42.6	30.0	28.6	±5.3

5.4.5.3 Figures

The temperatures recorded by all the internal and ambient dataloggers used in both experiments are shown in Figures 5.4.5.3 to 5.4.5.8. Each graph shows the temperatures for a single Man-Pig pair. The datalogger for Pig 1A mouth malfunctioned and is therefore not included in Figure 5.4.5.3. The ambient temperatures in the three graphs for Experiment 2 are the same, as only one ambient datalogger was used in this experiment.

The majority of internal body readings, especially the rectal ones, started at very low temperatures because the core temperature of many of the bodies was still very low, despite having been defrosted for 48 hours prior to the start of each experiment. The rectal probes are 15cm long, thereby reaching far into the torso. Once the ambient temperature was reached, many of the internal temperature recordings followed it, rising during the day and dropping overnight, especially in the first 3 to 4 days of each experiment. From about Day 4 onwards, however, the internal datalogger temperatures rose significantly above the ambient. This was most marked in Pig 1C mouth (Figure 5.4.5.5) and Pig 2A mouth (Figure 5.4.5.6). Even when the ambient temperature dipped at night, the internal body temperatures remained at a high, and fairly constant, temperature. In all the graphs, the Man mouth temperatures tended to reach a peak temperature fairly early in the Experiment, whereas the Man rectum temperatures took longer to reach their maximum and remained at a more constant level throughout the remainder of the experiment. The internal temperatures in Figure 5.4.5.8 for Man-Pig pair 2C were still well above the ambient temperatures, but followed the day-night pattern more closely than any other Man-Pig pair in either experiment.

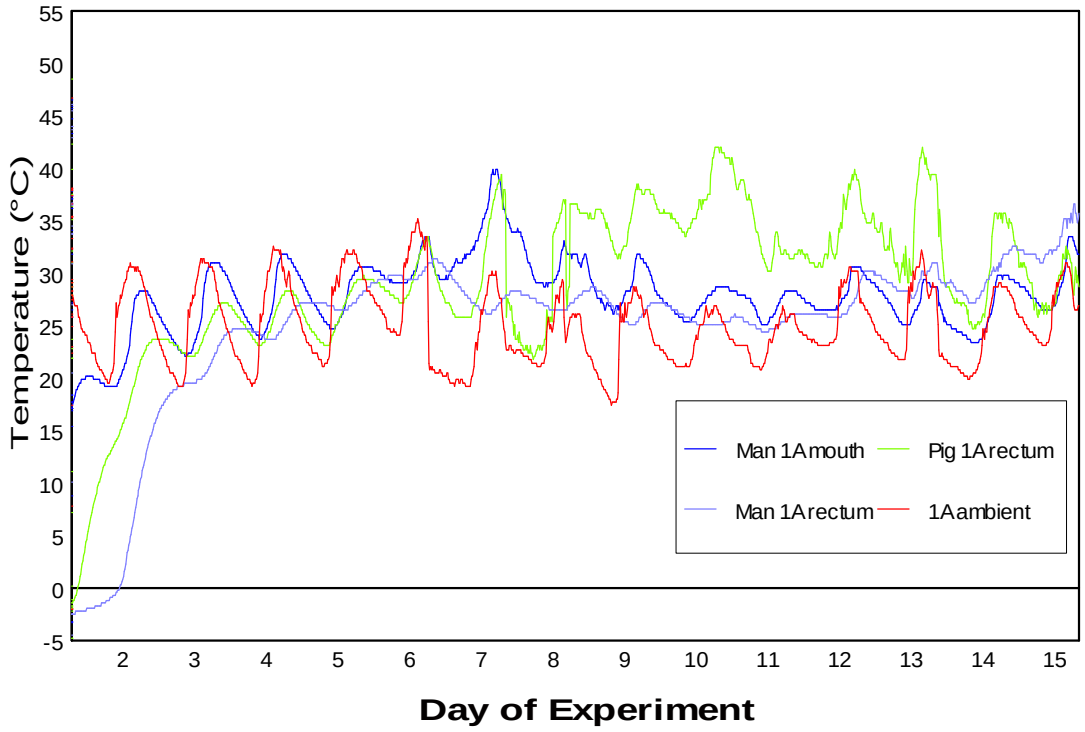


Figure 5.4.5.2 Ambient, mouth and rectal temperatures for Man 1A and Pig 1A (rectum only) from 19:00 on 16/07/08 (Day 1) to 18:30 on 30/07/08 (Day 15).

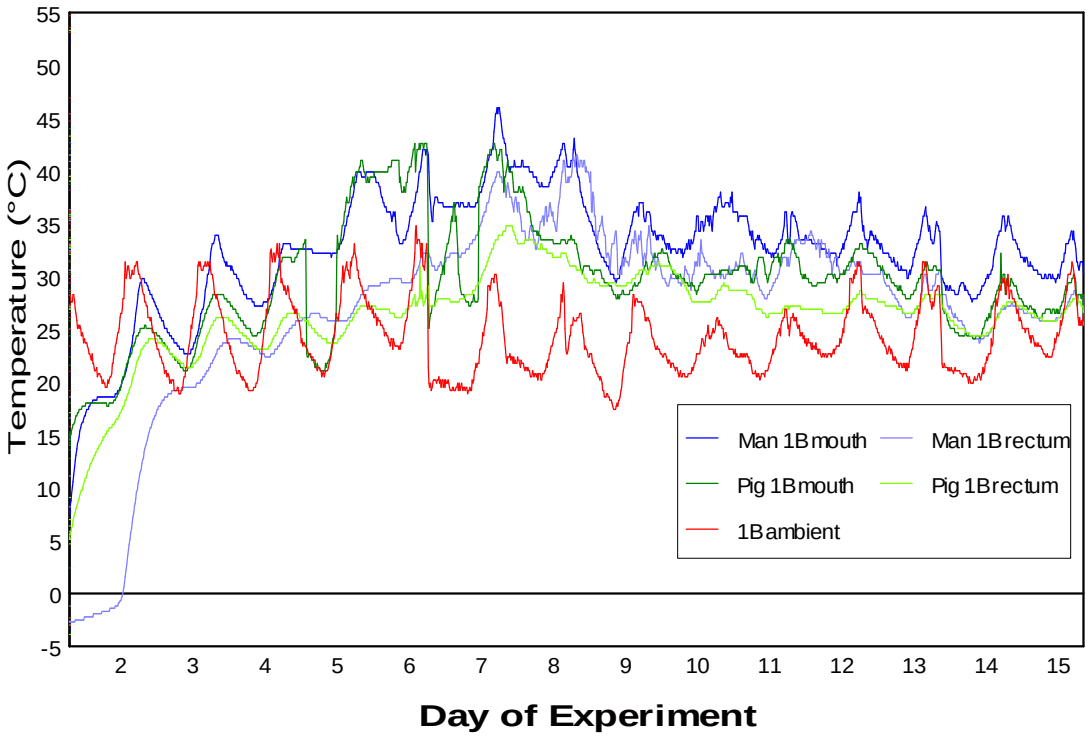


Figure 5.4.5.3 Ambient, mouth and rectal temperatures for Man 1B and Pig 1B from 19:00 on 16/07/08 (Day 1) to 18:30 on 30/07/08 (Day 15).

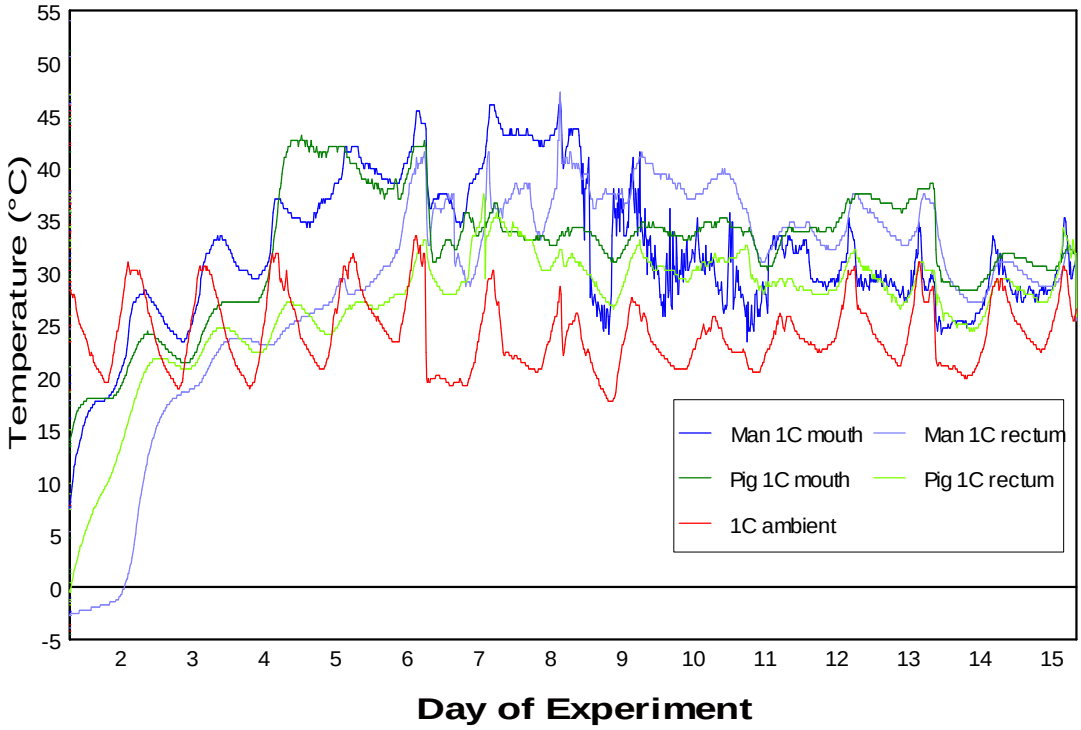


Figure 5.4.5.4 Ambient, mouth and rectal temperatures for Man 1C and Pig 1C from 19:00 on 16/07/08 (Day 1) to 18:30 on 30/07/08 (Day 15).

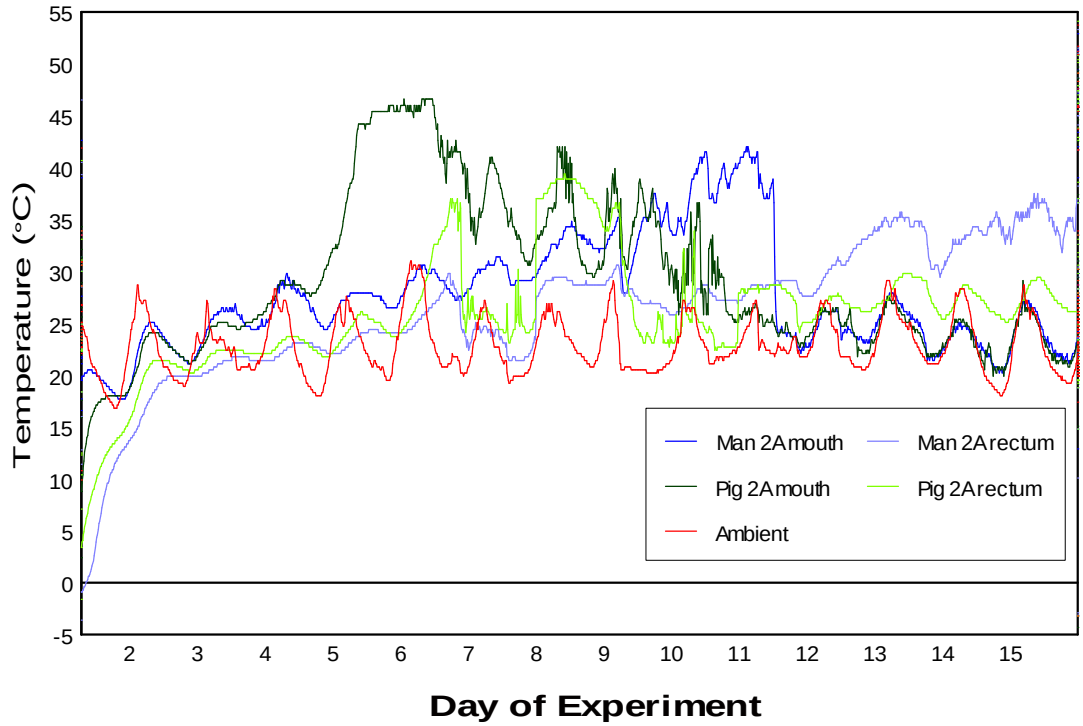


Figure 5.4.5.5 Ambient, mouth and rectal temperatures for Man 2A and Pig 2A from 19:00 on 20/07/09 (Day 1) to 10:00 on 04/08/09 (Day 16).

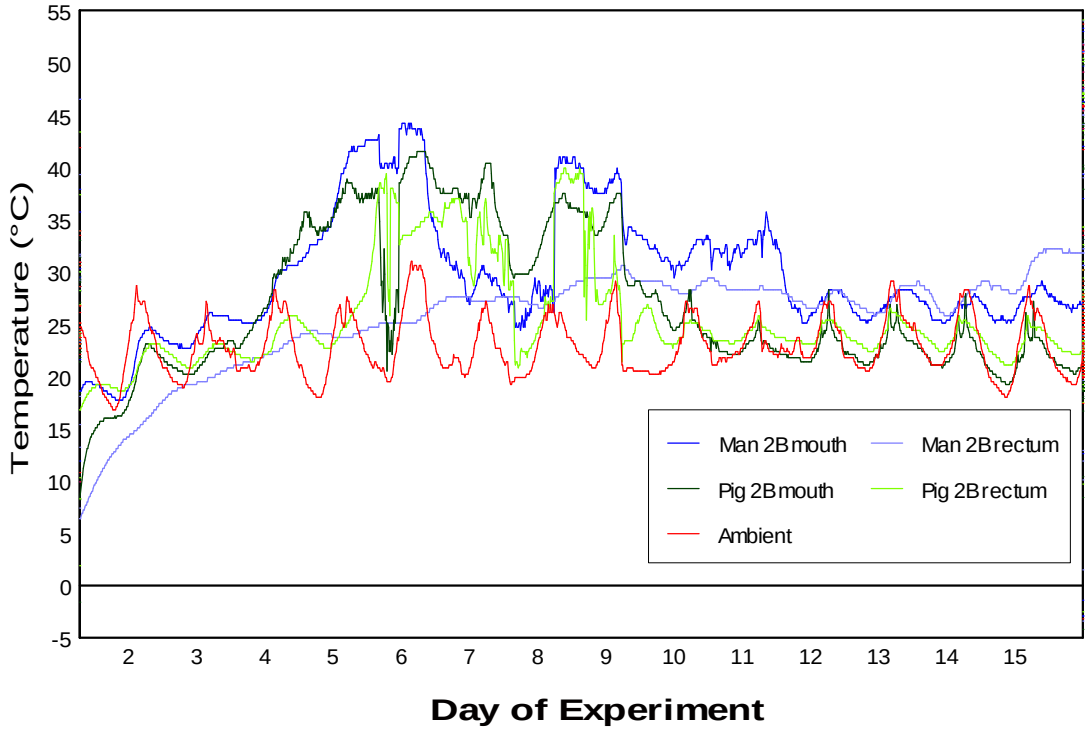


Figure 5.4.5.6 Ambient, mouth and rectal temperatures for Man 2B and Pig 2B from 19:00 on 20/07/09 (Day 1) to 10:00 on 04/08/09 (Day 16).

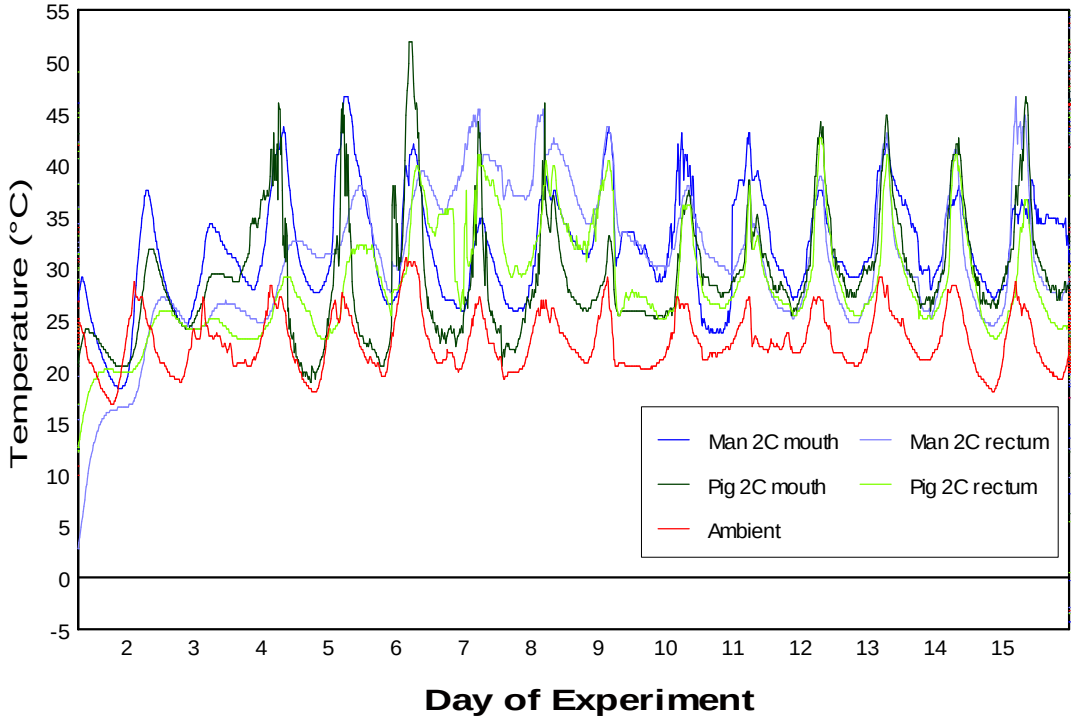


Figure 5.4.5.7 Ambient, mouth and rectal temperatures for Man 2C and Pig 2C from 19:00 on 20/07/09 (Day 1) to 10:00 on 04/08/09 (Day 16).

Figures 5.4.5.8 and 5.4.5.9 show the mean temperatures with 95% confidence intervals in each experiment, indicating that there was less variation around the mean for the ambient temperatures than for all the body internal temperatures, although the defrosting time will have contributed to this. All the internal mean temperatures were above the ambient mean temperatures, apart from Man 1A rectal temperatures. In contrast Man 1B and Man 1C mouth temperatures had the highest means, together with that of Pig 1C mouth.

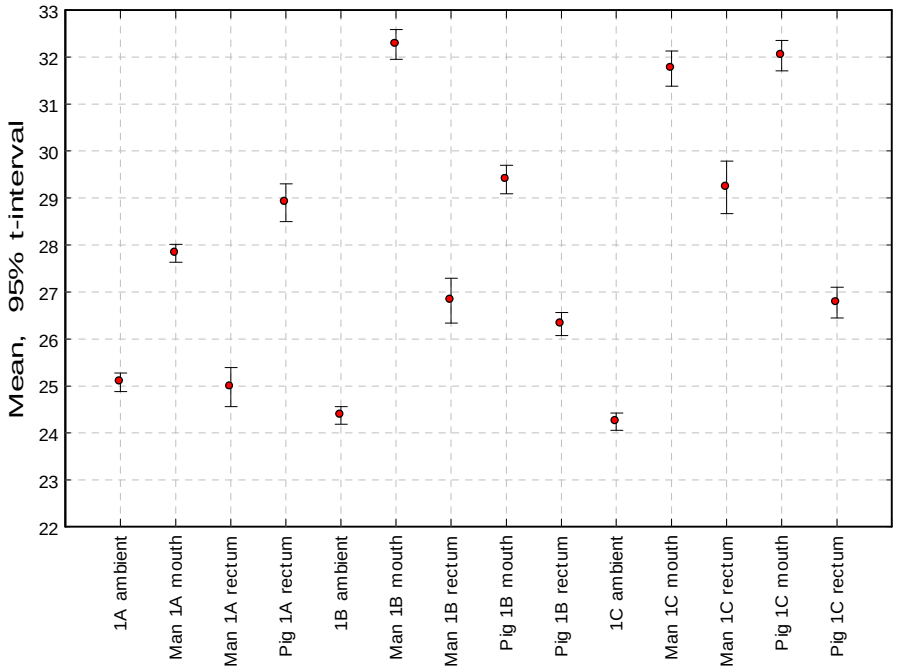


Figure 5.4.5.8 Mean temperatures with 95% confidence interval (Expt. 1)

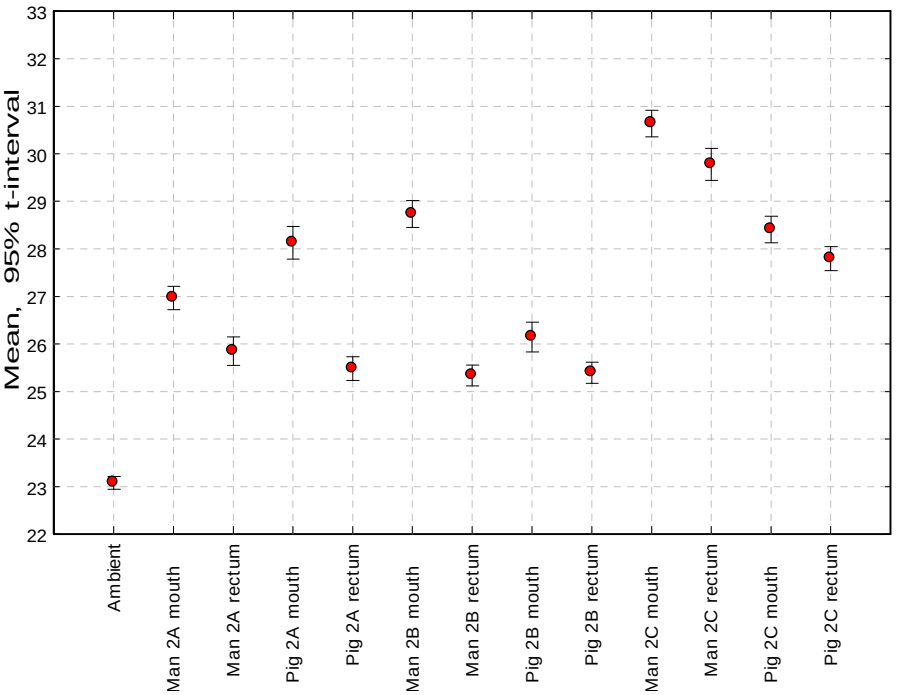


Figure 5.4.5.9 Mean temperatures with 95% confidence interval (Expt. 2)

5.4.6 Infrared temperature readings

The mean temperature above ambient recorded daily by infrared thermometer in the mouth and genitals of all 12 cadavers (i.e. Experiments 1 and 2 combined) are shown in Figure 5.4.6.1.

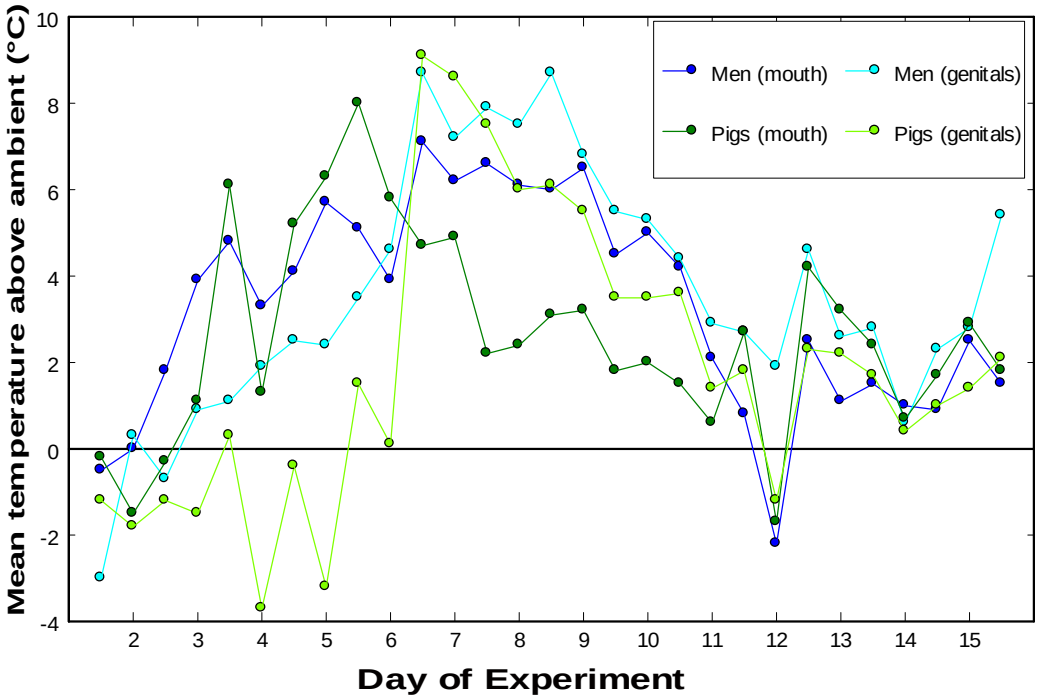


Figure 5.4.6.1 Mean temperatures above ambient for infrared readings on all 12 cadavers (Experiments 1 and 2)

On Day 2, the mean temperatures were up to 3°C below ambient. In both the men and the pigs, the temperatures in the mouth rose steeply and the genital temperatures lagged behind by about a day. The maximum temperatures reached were the genital temperatures for both men and pigs, at around 9°C above the ambient temperature on Day 7. The men's mouth temperatures also peaked on the same day, but at a lower temperature of just over 7°C. The pigs' mouth temperatures peaked at 8°C above ambient a day before, on Day 6. In general, the pig temperatures fluctuated more than the men temperatures, especially the pig genital temperatures between Days 4 and 6. The pig mouth temperatures decreased earlier than the other temperatures. The temperatures generally remained above the ambient for the duration of the study, except for a dip on Day 12 pm in all but the men genital temperatures.

5.4.7 Thermal imaging

A series of thermal images taken on Day 7 of Experiment 1, alongside a standard photograph, are shown in Figures 5.4.7.1 to 5.4.7.6.

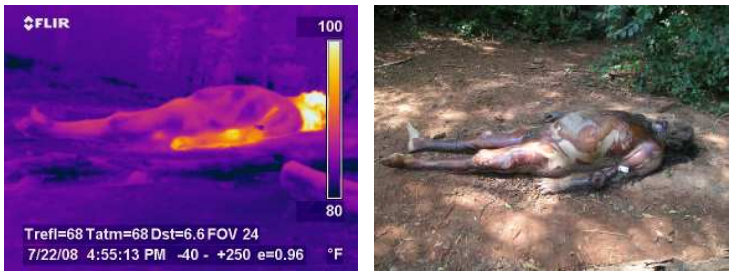


Figure 5.4.7.1 Man 1A

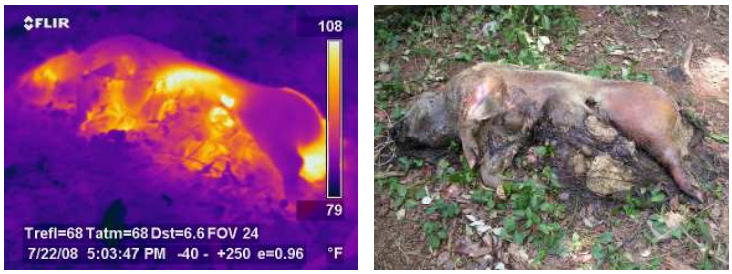


Figure 5.4.7.2 Pig 1A

Figure 5.4.7.3 Man 1B

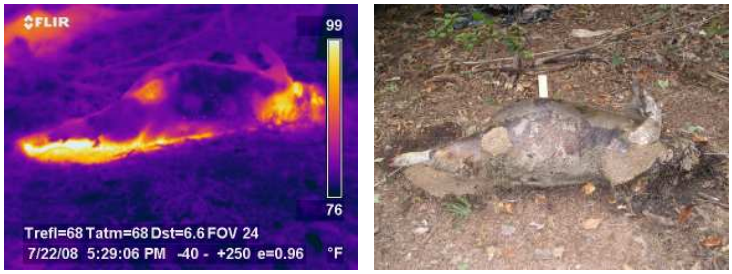


Figure 5.4.7.4 Pig 1B

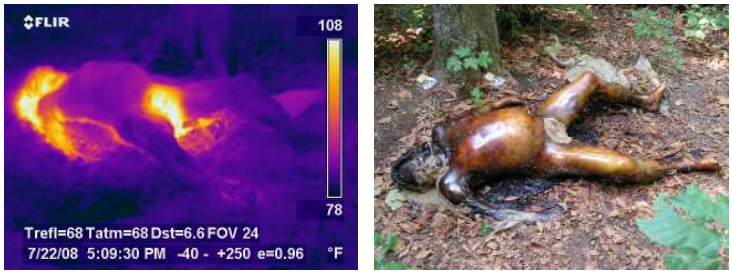


Figure 5.4.7.5 Man 1C

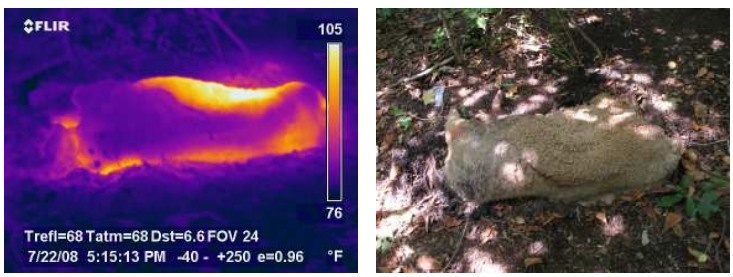


Figure 5.4.7.6 Pig 1C

Alongside each thermal image is a photograph of the same cadaver, taken on the same day and from approximately the same angle. In some of the photographs larval masses can clearly be seen which are also highly visible in the corresponding thermal image e.g. groin of Man 1B (Figure 5.4.7.3) and abdomen of Pig 1C (Figure 5.4.7.6). But in some photographs there is a larval mass visible, however the accompanying thermal image indicates that there is no significant heat associated with it, e.g. along the right arm of Man 1B (Figure 5.4.7.3) and across the back of Pig 1C (Figure 5.4.7.6). Some of the thermal images show larval masses that are not clearly visible in the photographs, e.g. the head of Man 1A (Figure 5.4.7.1), on the ground around Pig 1A (Figure 5.4.7.2) and around the head of Man 1C (Figure 5.4.7.5). In all the thermal images, the distribution of larval masses is comparable between men and pigs: in the head, along the underside of the body and in the genital area.

In order to compare the different cadavers, the maximum temperatures of larval masses recorded daily on each cadaver using thermal imaging are shown in Figure 5.4.7.7.

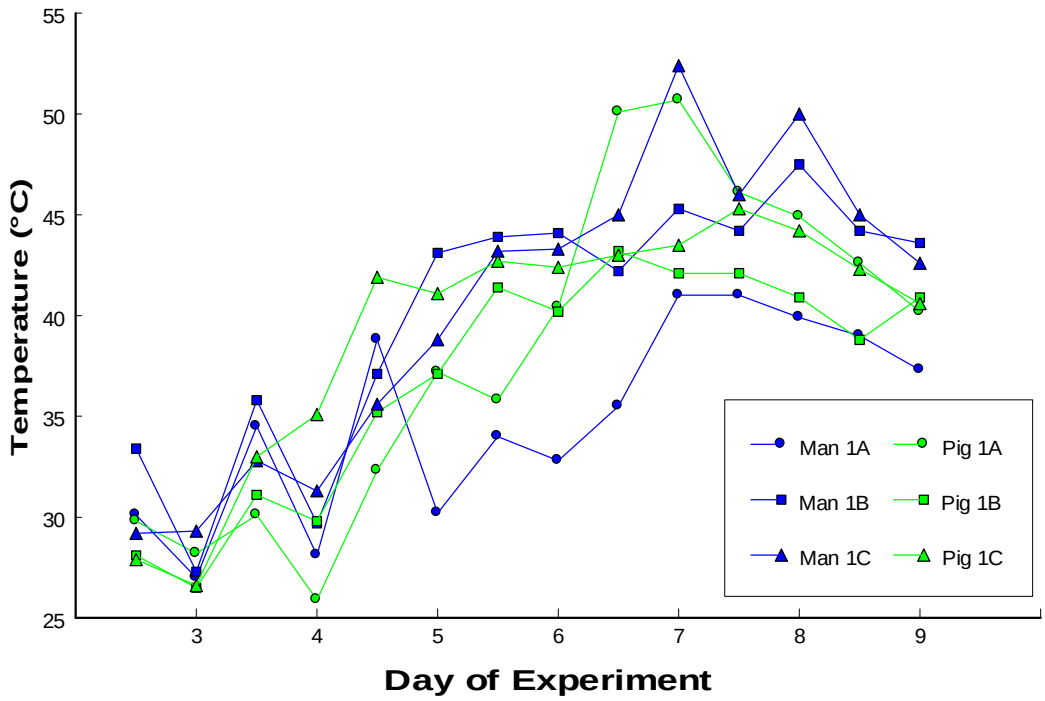


Figure 5.4.7.7 Maximum larval mass temperatures recorded using thermal imaging camera from Day 2 pm to Day 9 am.

From Day 2 to Day 5, there were larval masses starting in the mouth and then in the general head area; from Day 5 to Day 6 the larval masses were those around the general head area, either in/on the head itself, in the neck area or on the ground under the head; from Day 7 to Day 8 the larval masses shown in the graph were of the maximum larval mass recorded on each body ie. as well as the head area, also the genital area and under the body. Between Day 2 and Day 4 the temperatures appeared to fluctuate diurnally, in line with the ambient temperatures. From Day 4 until the morning of Day 7 the temperatures gradually increased to around 40°C to 45°C, before beginning to decrease that afternoon. In Pig 1A and Man 1C, the highest temperature reached over 50°C.

In order to counteract the diurnal effects of the ambient temperatures, the same data was plotted again, but using the maximum temperature above ambient temperature (Figure 5.4.7.8). This shows that the larval mass temperatures started below the ambient temperature, except for Man 1B which was around 3°C higher than the ambient. Thereafter, the temperatures gradually increased in a similar pattern to those shown in Figure 5.4.7.7, rising to almost 25°C above ambient temperature, before starting to decrease again on Day 7.

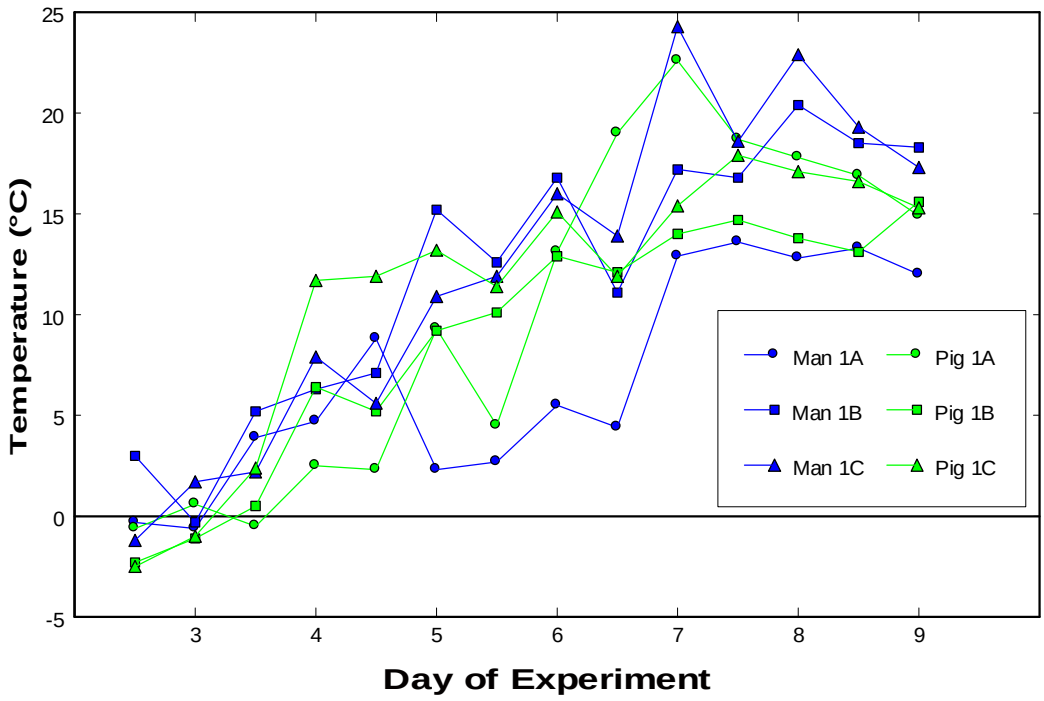


Figure 5.4.7.8 Maximum larval mass temperatures above ambient recorded using thermal imaging camera from Day 2 pm to Day 9 am.

5.5 Discussion

All cadavers used were first frozen, as it would not have been possible to obtain a number of fresh human cadavers to order. In order to ensure that the pigs and human cadavers were treated in the same way, the pigs were also frozen. Isiche *et al.* (1992) carried out studies on both fresh and previously frozen mouse carcasses and found no significant difference in larval load between them, suggesting that freezing carcasses and then defrosting prior to exposure does not affect their attractiveness to blowflies. In contrast, mouse carcasses aged prior to exposure yielded a progressively smaller larval load, as the amount of pre-exposure aging increased. Personal observations have also shown that human cadavers which have been stored for a period of time at temperatures >0°C, even in a mortuary fridge, do not attract blowflies as readily as fresh cadavers. Therefore freezing carcasses prior to exposure, rather than storing them unfrozen, has a lesser impact on their attractiveness to blowflies.

In all but one of the cadavers, oviposition by blowflies started on Day 1, within minutes of the bodies being laid out. Only on one human cadaver was oviposition delayed until Day 2. Female blowflies are under pressure to lay their eggs in moist, safe, nutritious areas of cadavers, to ensure optimal survival and growth rates and minimal predation and desiccation (Archer and Elgar, 2003). As expected, therefore, in all cadavers, eggs were laid primarily in the head area, including the main orifices of mouth, nose, ears and eyes. In addition, large numbers of eggs were also laid in the hairline of the humans and also in the fur, around the ears and snout, of the pigs. Oviposition on all cadavers also occurred between the cadavers and soil, and in the genital area, enabling female blowflies to continue to lay eggs in areas which are diminishing in quality (Archer and Edgar, 2033), but avoid competition with older larvae. In Experiment 1, eggs continued to be laid on Man-Pig pairs 1A and 1B throughout the experimental period, up to at least Day 15, whereas in all other Man-Pig pairs, oviposition ceased by Day 6 or 7.

In general, the pig cadavers attracted greater numbers of blowflies, although the species composition was the same between the men and pig cadavers. The percentage similarity of blowfly species was greater between each Man-Pig pair than between the men, the pigs or the pairs. Man-Pig pair 1A attracted smaller numbers of flies than Man-Pig pair 1B, but the most noticeable difference was in Man-Pig pair 1C, where the pig attracted much larger numbers of flies than the man. In retrospect, this was due to the location in which they were placed. Although all the men and pigs were placed within about 6m of their respective "pair", Man 3 was placed in an open area next to a path, as was Man 1 and Pig1, whereas Pig 3 was placed in a wooded area, as was Man 2 and Pig 2. Bornemissza (1956) stated that differences in microclimate, i.e. position of a carcass relative to trees and shrubs, influenced the rate of decay, and Reed (1958) found that insect populations were generally higher in wooded areas than in open ones, though higher temperatures in exposed areas counteracted potential differences in decomposition rate. Therefore it was the specific location in which the cadavers were placed which affected the numbers of blowflies attracted to them, rather than the species. Isiche *et al.* (1992) reported a higher Dipteral larval load on mouse carcasses placed in the shade, than those exposed to direct sunlight, but a greater number of species on the carcasses placed in sunlight. Conversely, studies carried out by Joy *et al.*, (2002) on racoon cadavers observed 1st instar *Phormia regina* larvae six hours earlier on sunlit cadavers than on shaded cadavers, and throughout the sampling period, the larvae sampled from the sunlit cadavers were consistently longer in length than those sampled from the shaded cadavers.

The thermal imaging carried out in Experiment 1 showed a very similar distribution of larval masses on both types of cadaver, with the head, genitals and under the bodies showing an increase in temperature over ambient, and therefore a significant larval load. The maximum larval mass temperatures ranged from 13°C to almost 25°C above ambient, with Man 1C and Pig 1A having the highest temperatures. Neither the men nor the pigs had higher larval mass temperatures overall, and neither did the placement of cadavers affect larval temperatures. Joy *et al*. (2002) also found no difference in larval temperatures between cadavers placed in sunlight or shade, but this was not

the case in a study carried out by Shean *et al*. (1993), who reported faster decomposition and higher larval mass temperatures on a sunlit pig carcass than on a shaded pig carcass. It is worth noting, however, that there was no replication of carcasses in this study and they were placed 600m apart. Likewise, Tantawi *et al*. (1998) stressed the importance of replicates in decomposition studies, when they found differences between fly species on rabbit carcasses.

There was some differential decomposition between all the cadavers. Most of the pigs exhibited a greater degree of larval load and decomposition, although Man 2C also decomposed to a near skeletal state by Day 16. The pigs tended to bloat more than the human cadavers, resulting in the splitting of their abdomens, and therefore exposed a larger surface area on which larvae could feed. Unlike the other human cadavers, the abdomen of Man 2C also split open, exposing the internal organs, and resulting in greater larval mass and more extensive larval feeding. Man 1A, by comparison, was still bloated by the end of the study on Day 15. Regardless of the state of decomposition of the cadavers, the larval developmental rate in Experiment 1 was similar between pigs and humans, with all larvae reaching 12-14mm in length. The development of larvae in the genitals of both the men and the pigs was delayed by approximately one day due to delayed oviposition in this area of the cadavers. The larvae reached their maximum size by the afternoon of Day 6, or morning of Day 7 (pig genitals). This growth rate supports studies carried out by Byrd and Allen (2001), who found that larvae of *Phormia regina* were ready to enter the pupariation stage after 6.5 days developing at a mean temperature of 25°C, a temperature comparable to that in this experiment which had a mean of 24.5°C over 15 days.

The PCA plots clearly show that there was no marked delineation between the two types of cadaver, but rather the cadavers are grouped in the Man-Pig pairs, indicating that location was a greater factor in any differences than cadaver type.

These results suggest that although there may be different rates of decomposition, with pig cadavers tending to have higher larval loads and faster decomposition rates, there is no significant difference between the species of blowfly which are attracted to pig and human cadavers, oviposition rates, nor to the development rate of the larvae. This supports the hypothesis that pigs are therefore a good surrogate for humans in entomology decomposition studies.

CHAPTER 6

DISCUSSION AND CONCLUSIONS

6.1 Discussion

The work presented here covered a number of areas of interest in forensic entomology and undertook to explore some areas of research which are less well studied or under-developed. I also endeavoured to employ new techniques to investigate more well-researched subjects, and took up the challenge of investigating an area of research which is crucial in forensic entomology studies, yet remains virtually untouched.

The primary use of forensic entomology is in estimating minimum time since death in order to aid the police and pathologists in cases of unexplained or untimely death (Wells & Lamotte, 2001). This requires knowing what species of insect are present on the body and/or at the scene, and what age they are. Most PMI estimates are calculated using baseline developmental data generated in controlled laboratory studies (Catts, 1992). However, there are many environmental factors which affect the development of blowflies, such as temperature, humidity, rainfall and sunlight. Therefore it is important to replicate field studies using cadavers in order to validate this data. Thus a series of piglet cadavers were placed in a semi-rural locality, and the insect infestation and subsequent decomposition were recorded. The studies were carried out in May and August respectively, and as expected, were colonised by blowflies, the most predominant species being *Lucilia sericata*. Comparison with published data by Anderson (2000) indicated a similar PMI estimate. One unexpected observation was that in the August experiment, flies were seen on two of the four cadavers as soon as they were exposed, but two of the cadavers had no flies on them initially, although by the second day there were egg masses on all four cadavers.

The question of when the blowfly eggs are actually laid on the body is an important one, as forensic entomologists are employed to estimate minimum

time since death, i.e. when the female blowfly first laid her eggs on the body, rather than when the person actually died. If a body is fully exposed in the open air, in a warm environment, it is usually expected that adult blowflies will be attracted to the body within minutes and will begin to feed and the females lay eggs.

There are, however, important factors which may affect the rate of oviposition. It is generally thought that blowflies are fairly inactive at night (Tessmer et al., 1995), although it has been reported (Singh & Bharti, 2001), but it is unlikely that oviposition can take place at night under natural conditions (Amendt *et al*., 2007). Temperature is also an important factor, as blowflies may only be active and oviposit above a certain ambient temperature (Erzinçlioğlu, 1986). An important factor is the accessibility of the body to blowflies. If a body has been wrapped, enclosed, submerged, buried or made inaccessible in some way, oviposition may be severely delayed. One such scenario, common in forensic entomology casework, is when a cadaver is discovered indoors. Research has shown a delay in oviposition on indoor bodies of 24 hours on seven out of nine piglet cadavers (Reibe & Madea, 2010) and five days (Anderson, 2011). In both studies, the indoor piglets had lower larval loads and therefore slower decomposition than the outdoor piglets.

These studies were replicated at the Natural History Museum in London, with five replicates of a pair of piglets, one placed indoors and one outdoors. These studies were carried out in different seasons and therefore in a range of ambient temperatures. Although there was a delay in oviposition on the indoor piglet in two of the five replicates, oviposition did take place on the same day in two of the replicates, and in one replicate, oviposition was delayed on the outdoor pig by nine days. In one of the experiments, the indoor piglet decomposed much faster than the outdoor piglet, due to a severe drop in outdoor ambient temperature. These results illustrate the importance of carrying out replicate experiments during different seasons, as temperature can be as great a barrier to oviposition as physical factors.

In some cases the location of the body may result not just in a delay in blowfly oviposition, but in a disruption of the expected order of insects. The study undertaken in Tennessee on a body in a bin resulted in a significant delay in oviposition by blowflies, with Phorids colonising the body first, supporting Campobasso *et al*. (2004) who reported *Megaselia scalaris* on an exhumed body in Southern Italy, and suggested that although usually relegated to a secondary forensic role, when larger flies are prevented from colonizing a body due to reduced access, such as burial, Phorid species may occur in the early stages of decomposition. Phorids have been found present in cases in the UK, especially during winter when Calliphorids are less common (Disney, & Manlove, 2005; Manlove & Disney, 2007), suggesting that this is an area of research which warrants further study.

Aside from oviposition, another important factor to take into account when estimating minimum post-mortem interval is the temperature at which the larvae were developing. Late 2nd and 3rd instar larvae group together to form larval masses, raising the local temperature and enabling a faster rate of development (Hanski, 1977). The difference between ambient and larval mass temperature may be greater in colder climates, as there is an upper thermal limit, above which larvae will die. In these studies, larval masses reached no higher than 45°C, both in the UK and in the USA, despite the ambient temperatures being very different. Charabidze *et al.* (2011) suggested that heat emission can occur even with "small" masses if they are composed of high number of 2nd instar larvae. Studies carried out in the laboratory at the NHM on small numbers of *Calliphora vicina* larvae showed an increase in larval mass temperature of 1°C-4°C. These studies were fairly limited due to small numbers of replicates and the use of differing ratios of numbers of larvae to grams of food, introducing competition as an additional factor, however being able to quantify the exact number of larvae in a larval mass and the degree of temperature increase would be invaluable to practising forensic entomologists.

A new technique was employed in these studies to investigate the dynamics of larval masses. Thermal imaging is non-invasive, allowing larval mass temperatures to be measured and recorded without disturbing the larvae. This

is important not only to ensure that an accurate measurement is recorded, as larval masses lose their temperature quickly if disturbed, but in crime scene work a body should be interfered with as little as possible, and a probe could damage the body or leave a post-mortem wound. Another advantage to using thermal imaging is that the temperatures over a large area can be measured and recorded simultaneously, as opposed to an infrared datalogger where only a single spot is measured. In addition, thermal images can be stored, to be studied and analysed at a later date using sophisticated software, rather than relying simply on handwritten notes.

One disadvantage of thermal imaging, however, is that if the body surface is in direct sunlight, it may be difficult to differentiate between larval mass and body surface on the thermal image due to solar heating (Cianci & Sheldon, 1990). In these circumstances, it is preferable to carry out thermal imaging in the early morning or evening, when the body surface is still cool and there is a greater contrast between larval mass and skin surface temperatures. Larval mass temperatures only remain elevated as long as the mass is tightly packed (Slone and Gruner, 2007) therefore as soon as the larvae are disturbed, the larval mass temperature quickly falls towards the ambient temperature. This supports the suggestion that thermal imaging would be invaluable at crime scenes, especially where a body may be wrapped or covered and the temperatures cannot be taken using traditional methods without uncovering and disturbing the larval mass.

Slone and Gruner (2007) found that the hottest temperature in the larval mass was not necessarily in the centre of the mass, therefore they took a number of temperature readings in different regions of each mass. One of the advantages of using a thermal imaging camera, however, is that the entire larval mass can be photographed and the temperatures across it recorded in a single image, giving a much more accurate indication of the larval mass temperatures. Previous studies have shown that the size of a larval mass influences its temperature (Turner & Howard, 1992; Marchenko, 2001), and this was confirmed with similar studies carried out on thermal masses on humans in Tennessee, using a thermal imaging camera. Although there was a positive

effect between larval mass area and its temperature, there was greater variation of temperature in smaller larval mass sizes, perhaps because there is an upper thermal limit, above which the larvae die. Contrary to Slone & Gruner's (2007) suggestion that there are varying temperatures across a larval mass, (Rivers *et al.*, 2011) suggest that there is a temperature continuum, the hottest part of the mass being in the centre, with the temperatures cooling towards the periphery. In order to avoid over-heating, larvae move around in and out of the mass, which River's *et al*. (2011) referred to as frenetic activity. Further studies could be carried out to investigate this further, and thermal imaging would be the ideal method to use, in order to avoid disrupting the larvae.

Once blowfly larvae have finished feeding on the body, they leave the body to pupate, therefore in forensic cases the oldest insect evidence may not be on the body itself, but in the surrounding environment (Amendt *et al*., 2007). Therefore an understanding of the behaviour of post-feeding larvae is important in order to be able to locate and recover them. Few studies have addressed this important life stage, although it has been suggested that larvae move in a specific direction (Tessmer & Meek, 1996), that they sometimes follow a narrow trail (Arnott & Turner, 2008), or that movement off a body is random (Gomes & Von Zuben, 2005). Observations of larval dispersal from human cadavers in Tennessee demonstrated that there are a number of variables involved, including substrate, distance, direction light levels, and time-span. The latter will be affected by the oviposition rates, i.e. if egg-laying occurred over a number of days, the dispersal of post-feeding larvae may also occur over a number of days, such as the larval dispersal from one body in Tennessee which continued for seven days. Kočárek (2001) suggested that larvae disperse from a body at night, in order to avoid predation, a behaviour which observations in Tennessee would support, as larvae were seen dispersing from bodies early in the morning on a number of occasions. Clearly post-feeding larval dispersal is a topic which warrants further examination, as any time spent in the post-feeding stage must be accounted for when making PMI estimations (Arnott & Turner, 2008).

Once the adult blowflies have emerged, the empty pupal cases remain in the environment, and these may also be recovered, identified and an analysis carried out to estimate a minimum post-mortem interval based on a single generation. They first have to be found, however, and apart from having been recovered from archaeological remains, it is unclear as to how long they may remain in the environment before they disintegrate, although Nuorteva (1987) suggested they may still be recovered after four years. This six year study carried out in the UK supports the view that the puparia will remain intact for some time, as they were easily recovered up to three and a half years after placement, but became disintegrated and more difficult to locate and recover after that time. A number of factors were taken into account, such as position along the transect and season, but the main contributing factor appeared to be length of time. In addition, puparia which were originally placed on the surface of the ground were less likely to remain intact over an extended period of time. This study was the first of its kind, although there were limitations to its design, such as whether the results would be applicable in a location with different environmental variables, such as vegetation or soil type.

Carrying out forensic casework sometimes raises more questions than it answers, but without doubt there can be no limit to the benefit gained from carrying out experimental work on human cadavers. In crime scene work, the body is seen at a specific point in time, and the collection of insect evidence must be carried out efficiently and non-invasively. Therefore enormous experience was gained by conducting forensic entomology studies in Tennessee on human cadavers. The only downside of this work is that the numbers of cadaver available to use are limited, so it is difficult to get adequate replicates for experimental work.

Much work has been carried out over the years on different types of animal carcass, ranging from birds to reptile to mammals. But the most commonly used animal cadaver is the pig, which resembles humans physically and physiologically, and which is also economical and ethical to use in scientific studies. The courts, however, are starting to question whether there is scientific validity in substituting non-human carcasses for human cadavers in forensic

entomology studies (Catts & Goff,1992). A single study was carried out using two pigs and one human cadaver (Schoenly *et al*., 2007), but with no replicates and no analysis of blowfly development, the authors stated that their study bolstered support for the pig-as-surrogate claim, but were unable to say whether PMI statistics differed between pig and human remains. Since then, no other study has examined the issue of whether pigs are a good model for humans. The study carried out as part of this research not only managed to address this issue, but also used six replicates of each cadaver type, ensuring that the results were robust rather than simply observational. Despite variations between the different cadavers, the differences were due to location of the bodies, i.e. experimental design, rather than to any real difference between the pigs and men. The results of this study give support to, and indeed validate, the current assumption that pigs are a good model substitute for humans in forensic entomology studies.

6.2 Conclusions

Much of the work presented in this thesis is novel in the field of forensic entomology, and the outcomes have contributed in the following ways:

- A small larval mass, containing as few as 20 larvae, may result in an increase of the temperature above ambient, whereas a mass of fewer larvae are unable to sustain an increase in temperature.
- While the rate of development of larvae in a reduced food resource is no different to that of larvae with an abundant resource, the resulting pupae and adults are significantly smaller.
- Thermal imaging is a novel technique which can be used effectively to measure the surface temperature of larval masses non-invasively and over a period of time.
- *Calliphora vicina* was the predominant blowfly species in an urban environment, whereas *Lucilia sericata* was the predominant species in a rural environment.
- Oviposition by female blowflies may be delayed on indoor cadavers, but if the cadaver is accessible to blowflies, e.g. through an open window,

then oviposition may take place at the same time as carcasses laid outdoors. However, even if oviposition occurs simultaneously on both indoor and outdoor carcasses, the ambient temperatures (indoors and outdoors) will subsequently determine the rate of development of blowfly larvae on both internal and external carcasses.

- Replicates of carcasses in a natural environment will not always be oviposited on simultaneously, and will not always decompose at the same rate.
- Empty pupal cases of *Calliphora vicina* stayed intact for up to three and a half years in a woodland environment, being more easily located if they had been buried rather than placed on the surface, and if they were searched for when the soil was dryer, usually in the summer months.
- Research on human cadavers may be restricted by the availability of fewer replicates, but the benefits of studying the complete decomposition process, which is not possible when carrying out casework, is invaluable.
- Reduced accessibility of human cadavers, i.e. in closed wheelie bins, severely disrupts the usual succession of insects, with Phorids being the first Diptera to gain access to the cadaver, and Calliphorid infestation being delayed by up to 10 days.
- The use of thermal imaging enabled the surface temperatures of an entire human body to be measured and recorded in a single frame, indicating the location of larvae not visible externally, a technique which could be valuable in forensic cases with wrapped, concealed or buried cadavers.
- The temperature of individual blowfly larvae also could be measured using thermal imaging. A positive relationship was demonstrated between the size and temperature of larval masses, although larval masses with a smaller surface area had a greater temperature range than those with a larger area.
- Observations of the dispersal of post-feeding Calliphorid larvae demonstrated that there may be considerable variation in the direction, distance and duration of larval dispersal.

- Studies comparing human with pig cadavers demonstrated that the same species of blowfly are attracted to both types of cadaver, ovipositing in the same body regions and developing at a similar rate. The main contributing factor to differences between cadavers was due to the placement locality, rather than to the species of cadaver. These results support the general assumption that pigs are a good model for humans in forensic entomology studies.

The field of forensic entomology is extensive and with so many contributing factors to consider and so many ways in which it can be applied, there is still much to learn and many areas needing further research. Its frequent application to legal matters means there is a continuing need for robust scientific studies and contributions to the field.

REFERENCES

Amendt, J., Zehner, R. and Reckel, F. (2008) The nocturnal oviposition behavior of blowflies (Diptera: Calliphoridae) in Central Europe and its forensic implications. *Forensic Science International* **175**: 61-64.

Amendt, J., Campobasso, C. P., Gaudry, E., Reiter, C., LeBlanc, H. N. and Hall, M. J. R. (2007) Best practice in forensic entomology – standards and guidelines. *International Journal of Legal Medicine* **121**: 90-104.

Ames, C. and Turner, B. (2003) Low temperature episodes in the development of blowflies: implications for postmortem interval estimation. *Medical and Veterinary Entomology* **17**: 178.

Anderson, G. S. (1999) Wildlife forensic entomology: determining time of death in two illegally killed black bear cubs. *Journal of Forensic Sciences* **44**: 856-859.

Anderson, G. S. (2000) Minimum and maximum development rates of some forensically important Calliphoridae (Diptera). *Journal of Forensic Sciences* **45**: 824-832.

Anderson, G. S. (2011) Comparison of decomposition rates and faunal colonization of carrion in indoor and outdoor environments. *Journal of Forensic Sciences* **56**: 136-142.

Anderson, G. S. & VanLaerhoven, S. L. (1996) Initial studies on insect succession on carrion in Southwestern British Columbia. *Journal of Forensic Sciences* **41**: 617-625.

Archer, M. S. and Elgar, M. A. (2003) Female breeding-site preferences and larval feeding strategies of carrion-breeding Calliphoridae and Sarcophagidae (Diptera): a quantitative analysis. *Australian Journal of Zoology* **51**: 165-174.

Arnott S. and Turner, B. (2008) Post-feeding larval behaviour in the blowfly, *Calliphora vicina*: Effects on post-mortem interval estimates. *Forensic Science International* **177**: 162-167.

Ardő, P. (1953) "Likflugan, *Conicera tibialis* Schmitz, i Sverige (Dipt. Phoridae)." *Opuscula Entomologica* **18**: 33-36.

Ash, N. and Greenberg, B. (1975) Developmental temperature responses of the sibling species *Phaenicia sericata* and *Phaenicia pallescens*. *Annals of the Entomological Society of America* **68**: 197-200.

Barton Browne, L., Bartell, R. J. & Shorey, H. H. (1969) Pheromone-mediated behaviour leading to group oviposition in the blowfly, *Lucilia cuprina*. *Journal of Insect Physiology* **15**: 1003-1014.

Beaver, R. A. (1972) Ecological studies on Diptera breeding in dead snails. I. Biology of the species found in *Cepaea nemoralis* (L.). *The Entomologist* **105**: 41-52.

Beaver, R. A. (1977) Non equilibrium "island" communities: Diptera breeding in dead snails. *Journal of Animal Ecology* **46**: 783-798.

Benecke, M. and Lessig, R. (2001) Child neglect and forensic entomology. *Forensic Science International* **120**: 155-159.

Bornemissza, G. F. (1956) An analysis of arthropod succession in carrion and the effect of its decomposition on the soil fauna. *Australian Journal of Zoology* **5**: 1-12.

Byrd, J. H. & Allen, J. C. (2001) The development of the black blow fly, *Phormia regina* (Meigen). *Forensic Science International* **120**: 79-88.

Byrd, J. H. & Castner, J. L. (2001) *Forensic Entomology: The Utility of Arthropods in Legal Investigations*. CRC Press, Boca Raton.

Campobasso, C. P., Disney, R. H. L. and Introna, F. (2004) A case of *Megaselia scalaris* (Loew) (Dipt., Phoridae) breeding in a human corpse. *Aggrawal's Internet Journal of Forensic Medicine and Toxicology* **5**: 3-5.

Catts, E. P. (1992) Problems in estimating the post-mortem interval in death investigations. Journal of Agricultural Entomology 9: 245-255.

Catts, E. P. and Goff, M. L. (1992) Forensic entomology in criminal investigations. *Annual Review of Entomology* **37**: 253-272.

Campobasso, C. P., Gheradi, M., Caligara, M., Sironi, L. and Introna, F. (2004) Drug analysis in blowfly larvae and in human tissues. *International Journal of Legal Medicine* **118**: 210-214.

Charabidze, D., Bourel, B. and Gosset, D. (2011) Larval-mass effect: Characterisation of heat emission by necrophageous blowflies (Diptera: Calliphoridae) larval aggregates. *Forensic Science International* **211**: 61-66.

Cianci, T. J. and Sheldon, J. K. (1990) Endothermic generation by blow fly larvae *Phormia regina* developing in pig carcasses. *Bulletin of the Society of Vector Ecology* **15**: 33.

Clark, K., Evans, L. and Wall, R. (2005) Growth rates of the blowfly, *Lucilia sericata*, on different body tissues. *Forensic Science International* **156**: 145-149.

Coe, M. (1978) The decomposition of elephant carcasses in the Tsava (East) National Park, Kenya. *Journal of Arid Environments* **1**: 71-86.

Cornaby, B. W. (1974) Carrion reduction by animals in contrasting tropical habitats. *Biotropica* **6**: 51-63.

Cragg, J. B. (1955) The natural history of sheep blowflies in Great Britain. *Annals of Applied Biology* **42**: 197-207.

Crosby, T. K., Watt, J. C., Kistemaker, A. C. and Nelson, P. E. (1986) Entomological identification of the origin of imported cannabis. *Forensic Science Society* **26**: 35.

Davies, L. and Ratcliffe, G. G. (1994) Development rates of some pre-adult stages in Blowflies with reference to low-temperatures. *Medical and Veterinary Entomology* **8**: 245-254.

Dekeirsschieter, J., Verheggen, F. J, Gohy, M., Hubrecht, F., Bourguignon, L., Lognay, G. and Haubruge, E. (2009) Cadaveric volatile organic compounds released by decaying pig carcasses (*Sus domesticus* L.) in different biotopes. *Forensic Science International* **189**: 46-53.

Denno, R. F. and Cothran, W. R. (1975) Niche relationships of a guild of necrophagous flies. Annals of the Entomological Society of America **68**: 741-745.

Disney, R. H. L. and Manlove, J. D. (2005) First occurrences of the Phorid, *Megasalia abdita*, in forensic cases in Britain. *Medical and Veterinary Entomology* **19**: 489-491.

Dix, J. and Graham, M. (2000) *Time of death, decomposition and identification. An atlas.* CRC Press, Florida, USA.

Doenier, C. C. (1940) Carcass temperatures and their relation to winter blowfly populations and activity in the Southwest. *Journal of Economic Entomology* **33**: 166-170.

Donovan, S. E., Hall, M. J. R. H., Turner, B. D. and Moncrieff, C. B. (2006) Larval growth rates of the blowfly, *Calliphora vicina*, over a range of temperatures. *Medical and Veterinary Entomology* **20**: 106-114.

Early, M. and Goff, M. L. (1986) Arthropod succession patterns in exposed carrion on the island of O'Ahu, Hawaiian Islands, USA. *Journal of Medical Entomology* **23**: 520-531.

Easton, A. M. (1966) The Coleoptera of a dead fox (*Vulpes vulpes* (L.)); including two species new to Britain. *Entomologist's Monthly Magazine* **102**: 205-210.

Easton, A. M. and Smith, K. G. V. (1970) The Entomology of the Cadaver. *Medical Science Law* **10**: 208-215.

Ellison, G. T. H. (1990) The effect of scavenger mutilation on insect succession at impala carcasses in southern Africa. *Journal of Zoology (London)* **220**: 679-688.

Erzinçlioğlu, Z. (1996) *Blowflies*. Naturalists' Handbooks **23**. The Richmond Publishing Co. Ltd., Slough.

Erzinçlioğlu, Z. (1986) Areas of research in forensic entomology. *Medicine, Science and the Law* **26**: 273-278.

Faulkner, D. K. (1986) The mass burial: an entomological perspective. In: Donnan, C. P. & Cock, G. A. (Eds.) The Pacatmu papers 1. *Museum of Cultural History, University of California, Los Angeles*, pp. 145-150.

Gennard, D. E. (2007) *Forensic Entomology: An Introduction*. John Wiley & Sons Ltd, Chichester.

Gilbert, B. M. and Bass, W. M. (1967) Seasonal dating of burials from the presence of fly pupae. *American Antiquity* **32**: 534-535.

Godoy, W. A. C., Fowler, H. G., von Zuben, C. J., Ziti, L. and Ribeiro, O. B. (1995) Larval dispersion in *Chrysomya megacephala*, *C. putoria* and *Cochliomyia macellaria* (Diptera: Calliphoridae). *Journal of Applied Entomology* **119**: 264-266.

Godoy, W. A. C., von Zuben, C. J. and dos Reis, S. F. (1996) Larval dispersal in *Chrysomya megacephala*, *Chrysomya putoria* and *Cochliomyia macellaria* (Diptera: Calliphoridae) – Ecological implications of aggregation behaviour. *Journal of Applied Entomology* **120**: 423-426.

Goff, M. L. (1991) Comparison of insect species associated with decomposing remains recovered inside dwellings and outdoors on the island of Oahu, Hawaii. *Journal of Forensic Sciences* **36**: 748-753.

Goff, M. L. (1993) Estimation of postmortem interval using arthropod development and successional patterns. *Forensic science review* **5**: 81-94.

Goff, M. L. (2000) *A Fly for the Prosecution: How insect evidence helps solve crimes*. Harvard University Press, Cambridge, MA, USA.

Goff, M. L., Brown, W. A., Hewadikaram, K. A. and Omori, A. I. (1991) Effect of heroin in decomposing tissues on the development rate of *Boettcherisca peregrina* (Diptera, Scacophagidae) and implications of this effect on the estimation of post mortem intervals using arthropod development patterns. *Journal of Forensic Sciences* **36**: 537-542.

Goff, M. L., Omori, A. I. and Goodbrod, J. R. (1989) Effect of cocaine in tissues on the development rate of *Boettcherisca peregrina* (Diptera: Sarcophagidae). *Journal of Medical Entomology* **26**: 91-93.

Gomes, L. and von Zuben, C. J. (2005) Postfeeding radial dispersal in larvae of *Chrysomya albiceps* (Diptera: Calliphoridae): implications for forensic entomology. *Forensic Science International* **155**: 61-64.

Goodbrod, J. R. and Goff, M. L. (1990) Effects of larval population density on rates of development and interactions between two species of *Chrysomya* (Diptera: Calliphoridae) in laboratory culture. *Journal of Medical Entomology* **27**: 338-343.

Grassberger, M. and Reiter, C. (2001) Effect of temperature on *Lucilia sericata* (Diptera: Calliphoridae) development with special reference to the Isomegalen- and Isomorphen- diagram. *Forensic Science International* **120**: 32-36.

Green, A. A. (1951) The control of blow-flies infesting slaughterhouses. I. Field observations on the habits of blow-flies. *Annals of Applied Biology* **38**: 475-495.

Greenberg, B. (1990) Behavior of postfeeding larvae of some Calliphoridae and a Muscid (Diptera). *Annals of the Entomological Society of America* **83**: 1210-1214.

Greenberg, B. (1991) Flies as forensic indicators. *Journal of Medical Entomology* **28**: 565-577.

Greenberg, B. and Kunich, J. C. (2002) *Entomology and the Law: Flies as Forensic Indicators*. Cambridge University Press, Cambridge, UK.

Greenberg, B. and Tantawi, T. I. (1993) Different developmental strategies in two boreal blowflies (Diptera: Calliphoridae). *Journal of Medical Entomology* **30**: 481-484.

Hall, R. D. (1990) Medicocriminal Entomology, in *Entomology and Death: A Procedural Guide*. Eds. Catts, E. P. & Haskell, N. H. Joyce's Print Shop, Clemson, S. C.

Hall, R. D. (2001) Introduction: Perceptions and Status of Forensic Entomology. *Forensic Entomology: The Utility of Arthropods in Legal Investigations*. Eds. Byrd, J. H. & Castner, J. L. CRC Press, Boca Rotan.

Hall, R. D. and Doisy, K. E. (1993) Length of time after death: effect on attraction and oviposition or larviposition of midsummer blow flies (Diptera, Calliphoridae) and flesh flies (Diptera, Sarcophagidae) of medicolegal importance in Missouri. *Annals of the Entomological Society of America* **86**: 589-593.

Hall, M., Whitaker, A. and Richards, C. (2012) Forensic Entomology. *Forensic Ecology Handbook: From Crime Scene to Court*. Eds. Márquez-Grant, N. and Roberts, J. Wiley-Blackwell. John Wiley & Sons, West Sussex.

Hall, M. J. R., Hutchinson, R. A., Farkas, R., Adams, Z. J. O. and Wyatt, N. P. (2003). A comparison of Lucitraps® and sticky targets for sampling the blowfly *Lucilia sericata*. *Medical and Veterinary Entomology* **17**: 280-287.

Hanski, I. (1976) Breeding experiments with carrion flies (Diptera) in natural conditions. *Annales Entomologici Fennici* **42**: 113-121.

Hanski, I. (1977) An interpolation model of assimilation by larvae of the blowfly *Lucilia illustris* (Calliphoridae) in changing temperatures. *Oikos* **28**: 187-195.

Hanski, I. and Kuusela, S. (1977) An experiment on competition and diversity in the carrion fly community. *Annales Entomologici Fennici* **43**: 108-115.

Hart, A. J., Whitaker, A. P. and Hall, M. J. R. (2008) The use of forensic entomology in criminal investigations: How it can be of benefit to SIOs. *The Journal of Homicide and Major Incident Investigation* **4**: 37-47.

Haskell, N.H. (2000) Testing reliability of animal models in forensic entomology with 50-200 lb. pigs vs. humans in Tennessee. *XXI International Congress of Entomology*, Foz do Iguassu, Brasil.

Hedouin, V., Bourel, B., Martin-Bouyer, L., Becart, A., Tournel, G., Deveaux, M. and Gosset, D. (1999a) Determination of drug levels in larvae of *Lucilia sericata* (Diptera, Calliphoridae) reared on rabbit carcasses containing morphine. *Journal of Forensic Sciences* **44**: 351-353.

Hedouin, V., Bourel, B., Martin-Bouyer, L., Becart, A., Tournel, G., Deveaux, M. and Gosset, D. (1999b) Morphine perfused rabbits - a tool for experiments in forensic entomotoxicology. *Journal of Forensic Sciences* **44**: 347-350.

Henssge, C., Madea, B. and Gallenkemper, E. (1988) Death time estimation in case work. II. Integration of different methods. *Forensic Science International* **39**: 77-87.

Hewadikaram, K. A. and M. L. Goff (1991) Effect of carcass size on rate of decomposition and arthropod succession patterns. *American Journal of Forensic Medicine and Pathology* **12**: 235-240.

Hwang, C. & Turner, B. D. (2005) Spatial and temporal variability of necrophagous Diptera from urban to rural areas. *Medical and Veterinary Entomology* **19**: 379-391.

Ireland, S. and Turner, B. (2006) The effects of larval crowding and food type on the size and development of the blowfly, *Calliphora vomitoria*. *Forensic Science International* **159**: 175-181.

Ives, A. R. (1991) Aggregation and coexistence in a carrion fly community. *Ecological Monographs* **6**: 75-94.

Ireland, S. and Turner, B. (2006) The effects of larval crowding and food type on the size and development of the blowfly *Calliphora vomitoria*. *Forensic Science International* **159**: 175-181.

Isiche, J., Hillerton, J. E. and Nowell, F. (1992). Colonization of the mouse cadaver by flies in southern England. *Medical and Veterinary Entomology* **6**: 168-170.

Johl, H. K. and Anderson, G. S. (1996) Effects of refrigeration on development of the blow fly, *Calliphora vicina* (Diptera: Calliphoridae) and their relationship to time of death. *Journal of the Entomological Society of British Columbia* **93**: 93-98.

Joy, J. E., Herrell, M. L. and Rogers, P. C. (2002) Larval fly activity on sunlit versus shaded racoon carrion in southwestern West Virginia with special reference to the black blowfly (Diptera: Calliphoridae). *Journal of Medical Entomology* **39**: 392-397.

Kamal, A. S. (1958) Comparative study of thirteen species of sarcosaprophagous Calliphoridae and Sarcophagidae (Diptera). *Annuals of the Entomological Society of America* **51**: 261-271.

Kaneshrajah, G. and Turner, B. (2004) *Calliphora vicina* larvae grow at different rates on different body tissues. *International Journal of Legal Medicine* **118**: 242-244.

Keiper, J. B., Chapman, E. G. and Foote, B. A. (1997) Midge larvae (Diptera: Chironomidae) as indicators of postmortem submersion interval of carcasses in a woodland stream: a preliminary report. *Journal of Forensic Sciences* **42**: 1074-1079.

Kempton, R. A. (1979) The structure of species abundance and measurement of diversity. *Biometrics* **35**: 307-321.

Kneidel, K. A. (1984) Influence of carcass taxon and size on species composition of carrion-breeding Diptera. *The American Midland Naturalist* **111**: 57-63.

Kočárek, P. (2001) Diurnal patterns of postfeeding larval dispersal in carrion blowflies (Diptera: Calliphoridae). *European Journal of Entomology* **98**: 117-119.

Lane, R. P. (1975) An investigation into blowfly (Diptera Calliphoridae) succession on corpses. *Journal of Natural History* **9**: 581-588.

LeBlanc, H. N. (2008) Olfactory stimuli associated with the different stages of vertebrate decomposition and their role in the attraction of the blowfly *Calliphora vomitoria* (Diptera: Calliphoridae) to carcasses. PhD thesis. University of Derby.

Leopold, R. A. (2000) Short-term cold storage of house-fly (Diptera: Muscidae) embryos: survival and quality of subsequent stages. *Annals of the Entomological Society of America* **93**: 884-889.

Lord, W. D. and Burger, J. F. (1984a) Arthropods associated with Herring Gulls (*Larus argentatus*) and Great Black-backed Gulls (*Larus marinus*) carrion on islands in the gulf of Maine. *Environmental Entomology* **13**: 1261-1268.

Lord, W. D. and Burger, J. F. (1984b) Arthropods associated with harbor seal (*Phoca vitulina*) carcasses stranded on islands along the New England coast. *International Journal of Entomology* **26**: 1282-1285.

Lord, W. D. and Stevenson, J. R. (1986) *American Registered Professional Entomologists*. Chesapeake Chapter: Washington DC.

Manlove, J. D. and Disney, R. H. L. (2007) The use of *Megaselia abdita* (Diptera: Phoridae) in forensic entomology. *Forensic Science International* **175**: 83-84.

Marchenko, M. I. (2001) Medico-legal relevance of cadaver entomofauna for the determination of the time of death. *Forensic Science International* **120**: 89-109.

Megnin, J. P. (1894) *La Faune des Cadavres: application de l'entomologie a la medecine legale*. Paris, G. Masson and Gautier-Villars.

Motter, M. G. (1898) A contribution to the study of the fauna of the grave. A study of one hundred and fifty disinterments, with some additional experimental observations. *Journal of the New York Entomological Society* **6**: 201-237.

Myskowiak, J.-B. and Doums, C. (2002) Effects of refrigeration on the biometry and development of *Protophormia terraenovae* (Robineau Desvoidy) (Diptera: Calliphoridae) and its consequences in estimating post-mortem interval in forensic investigations. *Forensic Science International* **125**: 254-261.

Nolte, K. B., Pinder, R. D. and Lord, W. D. (1992) Insect Larvae Used to Detect Cocaine Poisoning in a Decomposed Body. *Journal of Forensic Sciences* **37**: 1179-1185.

Nuorteva, P. (1987) Empty puparia of *Phormia terraenovae* R.-D. (Diptera Calliphoridae) as forensic indicators. *Annales Entomologici Fennici* **33**: 53-56.

O'Brien, C. and Turner, B. (2004) Impact of Paracetamol on *Calliphora vicina* larval development Entomology. *International Journal of Legal Medicine* **118**: 188-189.

Omar, B., Marwi, M. A., Sulaiman, S. and Oothuman, P. (1994) Dipteran succession in monkey carrion at a rubber tree plantation in Malaysia. *Tropical Biomedicine* **11**: 77-82.

Payne, J. A. (1965) A Summer Carrion Study of the Baby Pig *Sus scrofa* Linnaeus. *Ecology* **46**: 592-602.

Payne, J. A. and King, E. W. (1969) Lepidoptera associated with pig carrion. *Journal of the Lepidopterists Society* **23**: 191-195.

Payne, J. A. and King, E. W. (1970) Coleoptera Associated with Pig Carrion. *Entomologist's Monthly Magazine* **105**: 224-232.

Payne, J. A. and King, E. W. (1972) Insect succession and decomposition of pig carcasses in water. *Journal of the Georgia Entomological Society* **73**: 153-162.

Payne, J. A., King, E. W. and Beinhart, G. (1968a) Arthropod succession and decomposition of buried pigs. *Nature* **219**: 1180-1181.

Payne, J. A., Mead, F. W. and King, E. W. (1968b) Hemiptera associated with pig carrion. *Annals of the Entomological Society of America* **61**: 565-567.

Pickles, S., LeBlanc, H. & Hall, M. J. R. (2005) Living in squalor: when men and myiasis meet. 3rd Annual Meeting of the European Association of Forensic Entomology (EAFE), Lausanne, Switzerland.

Reed, H. B. (1958) A Study of Dog Carcass Communities in Tennessee, with Special References to the Insects. *The American Midland Naturalist* **59**: 213-245.

Reibe, S. and Madea, B. (2010) How promptly do blowflies colonise fresh carcasses? A study comparing indoor with outdoor locations. *Forensic Science International* **195**: 52-57.

Reiter, C. (1984) Zum wachstumsverhalten der maden der blauen schmeiβfliege *Calliphora vicina*. *Zeitschrift für Rechstmedizin* **91**: 295-308.

Rivers, D. B., Thompson, C. and Brogan, R. (2011) Physiological trade-offs of forming maggot masses by necrophagous flies on vertebrate carrion. *Bulletin of Entomological Research* **101**: 599-611.

Rodriguez, W. C. & Bass, W. M. (1983) Insect activity and its relationship to decay rates of human cadavers in East Tennessee. *Journal of Forensic Sciences* **28**: 423-432.

Rodriguez, W. C. & Bass, W. M. (1985) Decomposition of buried bodies and methods that may aid in their location. *Journal of Forensic Sciences* **30**: 836-852.

Roeterdink, E. M., Dadour, I. R. and Watling, R. J. (2004) Extraction of gunshot residues from the larvae of the forensically important blowfly Calliphora dubia (Macquart) (Diptera: Calliphoridae). *International Journal of Legal Medicine* **118**: 63-70.

Sadler, D. W., Chuter, G., Senevernatne, C. and Pounder, D. J. (1997) Commentary on: Barbiturates and analgesics in *Calliphora vicina* larvae. *Journal of Forensic Science* **42**: 1214-1215.

Sadler, D. W., Fuke, C., Court, F. and Pounder, D. J. (1995) Drug accumulation and elimination in *Calliphora vicina* larvae. *Forensic Science International* **71**: 191-197.

Samuel, W. M. (1988). The use of age classes of winter ticks on moose to determine time of death. *Canadian Society of Forensic Sciences Journal* **21**: 54-59.

Saunders, D. S. and Bee, A. (1995) Effects of larval crowding on size and fecundity of the blowfly, *Calliphora vicina* (Diptera: Calliphoridae). *European Journal of Entomology* **92**: 615-622.

Schoenly, K. G., Haskell, N. H., Hall, R. D. and Gbur, J. R. (2007) Comparative performance and complementarity of four sampling methods and arthropod preference tests from human and porcine remains at the Forensic Anthropology Center in Knoxville, Tennessee. *Journal of Medical Entomology* **44**: 881-894.

Shahid, S. A., Schoenly, K., Haskell, N. H., Hall, R. D. and Zhang, W. (2003) Carcass enrichment does not alter decay rates or arthropod community structure: a test of the arthropod saturation hypothesis at the Anthropology Research Facility in Knoxville, Tennessee. *Journal of Medical Entomology* **40**: 559-569.

Shean, B. S., Messinger, L. and Papworth, M. (1993) Observations of differential decomposition on sun exposed v. shaded pig carrion in coastal Washington State. *Journal of Forensic Science* **38**: 938-949.

Singh, D. and Bharti, M. (2001) Further observations on the nocturnal oviposition behaviour of blowflies (Diptera: Calliphoridae). *Forensic Science International* **120**: 124-126.

Slone, D. H. and Gruner, V. (2007) Thermoregulation in larval aggregations of carrion-feeding blow flies (Diptera: Calliphoridae). *Journal of Medical Entomology* **44**: 516-523.

Smith, K. G. V. (1975) The faunal succession of insects and other invertebrates on a dead fox. *Entomologist's Gazette* **26**: 277-287.

Smith, K. G. V. (1986) *A Manual of Forensic Entomology*. British Museum (Natural History) & London and Cornell University Press, Oxford.

Smith, K. E. & Wall, R. (1997) The use of carrion as breeding sites by the blowfly *Lucilia sericata* and other Calliphoridae. *Medical and Veterinary Entomology* **11**: 38-44.

Stafford, F. (1971) Insects of a medieval burial. *Science and Anthropology* **7**: 6-10.

Sullivan, T. P., Eaglstein, W. H., Davis, S. C. and Mertz, P. (2001) The pig as a model for human wound healing. *Wound Repair and Regeneration* **9**: 66–76.

Swift, B. (2010) Methods of time since death estimation within the early post-mortem interval. *The Journal of Homicide and Major Incident Investigation* **6**: 97-112.

Tantawi, T. I., Wells, J. D., Greenberg, B. and El-Kady, E. M. (1998) Fly larvae (Diptera: Calliphoridae, Sarcophagidae, Muscidae) succession in rabbit carrion: variation observed in carcasses exposed at the same time and in the same place. *Journal Of Egyptian German Society Of Zoology (Entomology)* **25**: 195-208.

Teskey, H. J. & Turnbull, C. (1979) Diptera puparia from prehistoric graves. *Canadian Entomologist* **111**: 527-528.

Tessmer, J. W. and Meek, C. L. (1996) Dispersal and distribution of Calliphoridae (Diptera) immature from animal carcasses in southern Louisiana. *Journal of Medical Entomology* **33**: 665-669.
Tessmer, J. W., Meek, C. L. & Wright, V. L. (1995) Circadian patterns of oviposition by necrophilous flies (Diptera: Calliphoridae) in southern Louisiana. *Southwestern Entomology* **20**: 665-669.

Turner, B. D. and Howard, T. (1992) Metabolic heat generation in dipteran larval aggregations: a consideration for forensic entomology. *Medical and Veterinary Entomology* **6**: 179-181.

Ullyet, G. C. (1950) Competition for food and allied phenomena in sheep blowfly populations. *Philosophical Transactions of the Royal Society of London Series B* **234**: 77-174.

Utsumi, K., Nakajima, M., Mitsuya, T. and Kaneko, K. (1958) Studies on the insects congregated to the albino rats died of different causes. *Ochanomizu Medical Journal* **7**: 119-129.

Vass, A. A., Bass, W. M., Wolt, J. D, Foss, J. E. and Ammons, J.T. (1992) Time since death determinations of human cadavers using soil solution. *Journal of Forensic Sciences* **37**: 1236–1253.

Villet, M. H., Richards, C. S. and Midgley, J. M. (2010). Chapter 7: Contemporary precision, bias and accuracy of minimum post-mortem intervals estimated using development of carrion-feeding insects. In *Current Concepts in*

Forensic Entomology (Eds. Amendt, J., Campobasso, C. P., Goff, M. L. and Grassberger, M.) Springer Science+Business Media B.V. pp. 109-137.

Wall, R., French, N. and Morgan, K. L. (1992) Effects of temperature on the development and abundance of the sheep blowfly Lucilia sericata (Diptera: Calliphoridae). *Bulletin of Entomological Research* **82**: 125-131.

Wells, J. D. & LaMotte, L. R. (2001) Estimating the Postmortem Interval. In *Forensic Entomology: The Utility of Arthropods in Legal Investigations* (Eds. Bryd, J. H. & Castner, J. L.) pp. 263-285. CRC Press, Boca Raton.

Appendix I Additional figures and tables

Data from Calliphorid puparia experiment from section 3.4.3

Table 3.4.3.1 Raw data, showing date of collection, number and percentage of puparia out of 40 recovered from each sample site, plus total percentage

Metre mark	Left marker		Centre marker		Right marker		Date collected	Mean percent
	Depth	No./40 = %	Depth	No./40 = %	Depth	No./40 = %		
1	Surface	0 = 0%	Leaf/soil	0 = 0%	Buried	17 = 42.5%	21.10.07	14.17%
2	Buried	35 = 87.5%	Surface	1 = 2.5%	Leaf/soil	5 = 12.5%	17.07.05	34.17%
3	Leaf/soil	0 = 0%	Buried	0 = 0%	Surface	0 = 0%	13.04.09	0.00%
4	Leaf/soil	1 = 2.5%	Surface	36 = 90%	Buried	4 = 10%	20.06.04	34.17%
5	Surface	0 = 0%	Buried	0 = 0%	Leaf/soil	0 = 0%	24.12.07	0.00%
6	Buried	25 = 62.5%	Leaf/soil	19 = 47.5%	Surface	13 = 32.5%	06.04.06	47.50%
7	Surface	19 = 47.5%	Leaf/soil	17 = 42.5%	Buried	28 = 70%	16.01.05	53.33%
8	Buried	0 = 0%	Surface	0 = 0%	Leaf/soil	0 = 0%	28.02.10	0.00%
9	Leaf/soil	28 = 70%	Buried	35 = 87.5%	Surface	8 = 20%	11.12.05	59.17%
10	Leaf/soil	22 = 55%	Surface	2 = 5%	Buried	10 = 25%	08.07.07	28.33%
11	Surface	28 = 70%	Buried	28 = 70%	Leaf/soil	29 = 72.5%	05.09.04	70.83%
12	Buried	0 = 0%	Leaf/soil	1 = 2.5%	Surface	0 = 0%	11.05.08	0.83%
13	Surface	5 = 12.5%	Leaf/soil	5 = 12.5%	Buried	2 = 5%	08.02.06	10.00%
14	Buried	24 = 60%	Surface	16 = 40%	Leaf/soil	10 = 25%	12.03.04	41.67%
15	Leaf/soil	0 = 0%	Buried	0 = 0%	Surface	0 = 0%	04.05.09	0.00%
16	Leaf/soil	3 = 7.5%	Surface	1 = 2.5%	Buried	1 = 2.5%	05.11.06	4.17%
17	Surface	36 = 90%	Buried	36 = 90%	Leaf/soil	35 = 87.5%	09.10.04	89.17%
18	Buried	0 = 0%	Leaf/soil	0 = 0%	Surface	0 = 0%	22.06.08	0.00%
19	Surface	2 = 5%	Leaf/soil	3 = 7.5%	Buried	1 = 2.5%	12.11.05	5.00%
20	Buried	0 = 0%	Surface	0 = 0%	Leaf/soil	0 = 0%	15.09.07	0.00%
21	Leaf/soil	23 = 57.5%	Buried	34 = 85%	Surface	23 = 57.5%	12.12.04	66.67%
22	Leaf/soil	2 = 5%	Surface	0 = 0%	Buried	11 = 27.5%	04.06.09	10.83%
23	Surface	19 = 47.5%	Buried	37 = 92.5%	Leaf/soil	20 = 50%	14.08.05	63.33%
24	Buried	0 = 0%	Leaf/soil	3 = 7.5%	Surface	4 = 10%	19.05.07	5.83%
25	Surface	34 = 85%	Leaf/soil	30 = 75%	Buried	37 = 92.5%	23.05.04	84.17%
26	Buried	0 = 0%	Surface	0 = 0%	Leaf/soil	0 = 0%	26.02.09	0.00%
27	Leaf/soil	36 = 90%	Buried	36 = 90%	Surface	30 = 75%	28.07.04	85.00%
28	Leaf/soil	0 = 0%	Surface	0 = 0%	Buried	0 = 0%	04.08.07	0.00%
29	Surface	3 = 7.5%	Buried	38 = 95%	Leaf/soil	33 = 82.5%	12.06.05	61.67%
30	Buried	0 = 0%	Leaf/soil	0 = 0%	Surface	0 = 0%	20.09.09	0.00%
31	Surface	8 = 20%	Leaf/soil	10 = 25%	Buried	34 = 85%	11.06.06	43.33%
32	Buried	0 = 0%	Surface	10 = 25%	Leaf/soil	0 = 0%	29.10.04	8.33%
33	Leaf/soil	0 = 0%	Buried	1 = 2.5%	Surface	0 = 0%	19.04.08	0.83%
34	Leaf/soil	3 = 7.5%	Surface	1 = 2.5%	Buried	0 = 0%	14.01.06	3.33%
35	Surface	0 = 0%	Buried	0 = 0%	Leaf/soil	0 = 0%	25.05.09	0.00%
36	Buried	39 = 97.5%	Leaf/soil	34 = 85%	Surface	5 = 12.5%	28.03.04	65%
37	Surface	16 = 40%	Leaf/soil	24 = 60%	Buried	1 = 2.5%	18.04.07	34.17%
38	Buried	0 = 0%	Surface	0 = 0%	Leaf/soil	0 = 0%	05.12.09	0.00%
39	Leaf/soil	37 = 92.5%	Buried	0 = 0%	Surface	24 = 60%	08.08.04	32.33%
40	Leaf/soil	0 = 0%	Surface	0 = 0%	Buried	0 = 0%	26.01.08	0.00%
41	Surface	0 = 0%	Buried	0 = 0%	Leaf/soil	0 = 0%	11.09.06	0.00%
42	Buried	36 = 90%	Leaf/soil	38 = 95%	Surface	25 = 62.5%	08.05.05	82.5%
43	Surface	0 = 0%	Leaf/soil	0 = 0%	Buried	0 = 0%	30.05.10	0.00%
44	Buried	1 = 2.5%	Surface	0 = 0%	Leaf/soil	0 = 0%	21.01.07	0.83%
45	Leaf/soil	32 = 80%	Buried	0 = 0%	Surface	31 = 77.5%	21.11.04	52.50%
46	Leaf/soil	0 = 0%	Surface	0 = 0%	Buried	0 = 0%	11.11.08	0.00%
47	Surface	3 = 7.5%	Buried	14 = 35%	Leaf/soil	30 = 75%	04.09.05	39.17%
48	Buried	0 = 0%	Leaf/soil	0 = 0%	Surface	0 = 0%	18.11.07	0.00%
49	Surface	10 = 25%	Leaf/soil	31 = 77.5%	Buried	20 = 50%	20.02.05	50.83%
50	Buried	0 = 0%	Surface	0 = 0%	Leaf/soil	0 = 0%	13.06.09	0.00%

Data from human vs. pig decomposition sticky traps from section 5.4.3

Table 5.4.3.1 Numbers of blowflies captured on sticky traps in Experiment 1

Species	Cadaver	Day 2-3	Day 4-5	Day 6-7	Day 8-9	Day 10-11	Day 12-13	Day 14-15
Cochliomyia macellaria	Control	1	0	2	0	5	1	0
	Man 1A	3	38	31	16	25	25	42
	Pig 1A	5	25	61	26	12	8	11
	Man 1B	3	5	13	9	6	1	7
	Pig 1B	2	11	10	0	2	1	1
	Man 1C	8	13	5	2	2	6	4
	Pig 1C	0	11	5	0	0	2	0
Lucilia illustris	Control	1	0	2	0	0	0	0
	Man 1A	4	11	3	0	3	0	0
	Pig 1A	4	37	4	0	0	0	0
	Man 1B	0	83	21	0	0	0	0
	Pig 1B	5	109	24	0	2	0	1
	Man 1C	3	5	5	0	0	0	0
	Pig 1C	19	12	3	0	0	0	0
Lucilia sericata	Control	0	0	0	0	1	1	0
	Man 1A	24	7	3	0	1	0	0
	Pig 1A	29	19	8	0	7	0	0
	Man 1B	13	32	1	2	1	1	0
	Pig 1B	17	35	8	0	0	0	1
	Man 1C	10	1	5	1	0	0	0
	Pig 1C	26	7	4	0	0	0	0
Phormia regina	Control	0	0	0	0	5	0	0
	Man 1A	11	36	17	6	8	8	3
	Pig 1A	13	19	25	11	15	3	7
	Man 1B	61	14	32	2	11	1	1
	Pig 1B	52	2	20	1	15	2	0
	Man 1C	32	9	8	4	1	0	0
	Pig 1C	38	17	2	3	0	1	0
Lucilia coeruleiviridis	Control	0	0	0	0	0	0	0
	Man 1A	27	32	1	0	0	0	0
	Pig 1A	17	58	11	1	1	0	3
	Man 1B	130	44	1	0	2	4	3
	Pig 1B	129	28	23	0	0	0	4
	Man 1C	22	9	0	0	5	1	0
	Pig 1C	130	12	2	0	3	0	0
Chrysomya rufifaces	Control	0	0	0	0	0	0	0
	Man 1A	0	10	5	0	2	2	4
	Pig 1A	2	25	0	0	0	0	0
	Man 1B	4	10	1	0	0	0	0
	Pig 1B	1	28	4	0	0	0	0
	Man 1C	2	7	0	0	0	0	0
	Pig 1C	1	13	0	0	0	0	0
Hermetia illucens	Control	0	0	0	0	0	0	0
	Man 1A	0	0	0	1	0	0	0
	Pig 1A	0	1	1	6	1	3	8
	Man 1B	0	0	1	12	9	5	11
	Pig 1B	0	0	0	2	1	5	1
	Man 1C	0	3	0	3	1	1	0
	Pig 1C	0	0	3	1	1	3	3

Sticky trap photographs from section 5.4.3.1

Figure 5.4.3.1 Sticky traps (Day 2-3) from Experiment 1

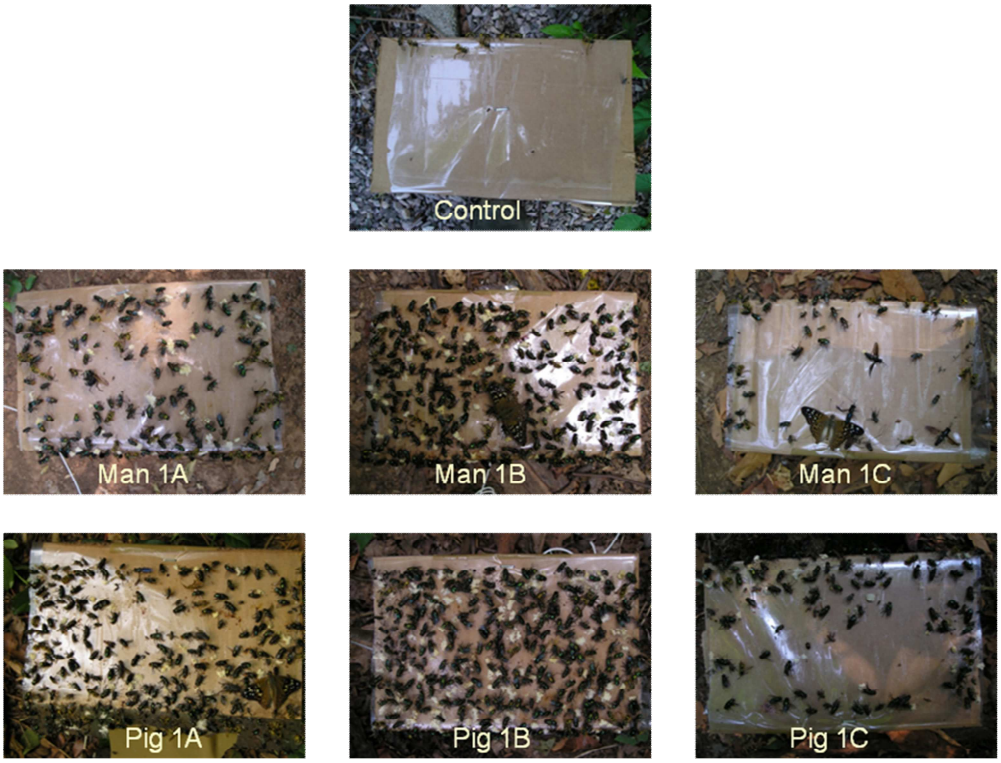


Figure 5.4.3.2 Sticky traps (Day 4-5) from Experiment 1

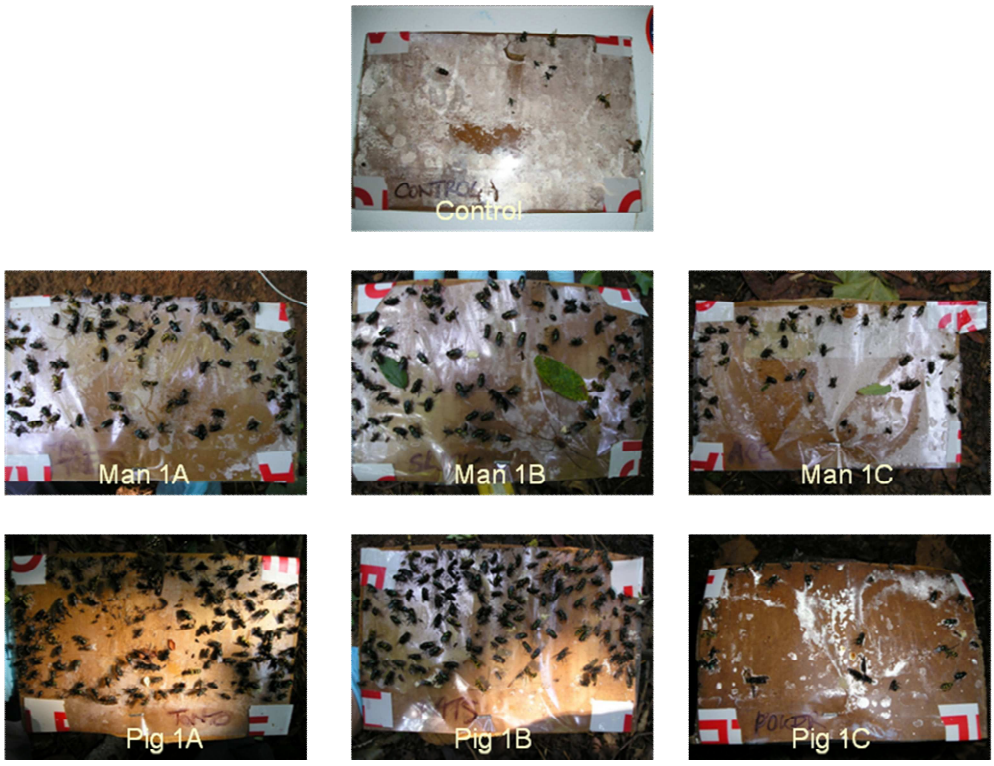


Figure 5.4.3.3 Sticky traps (Day 6-7) from Experiment 1

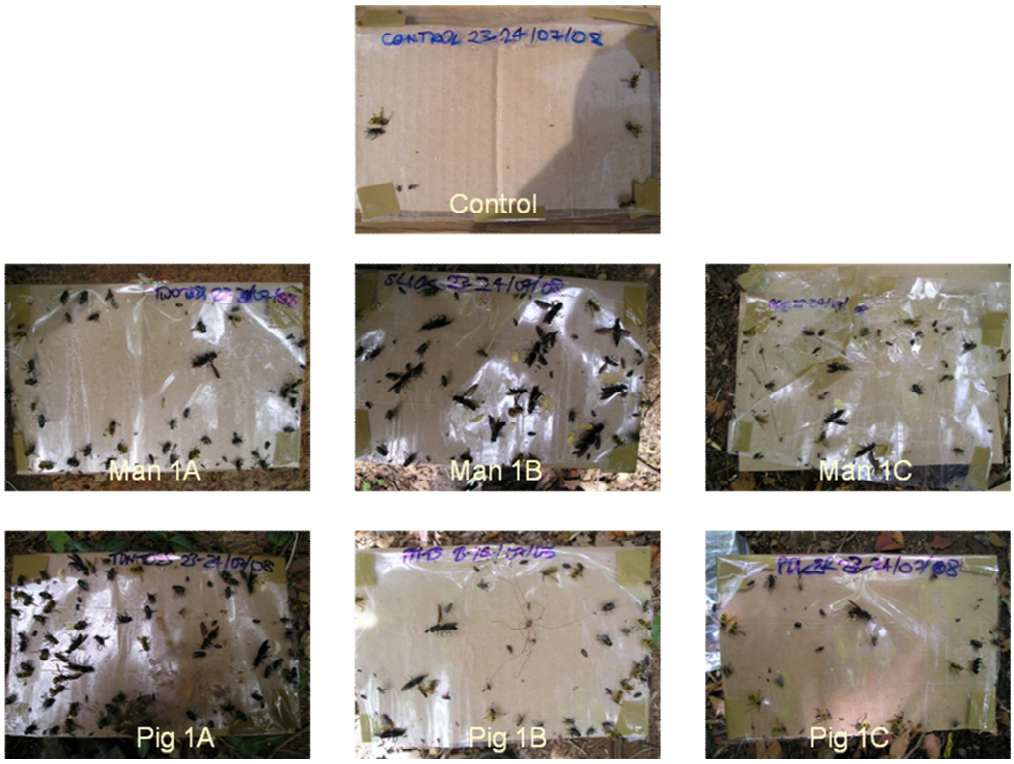


Figure 5.4.3.4 Sticky traps (Day 8-9) from Experiment 1

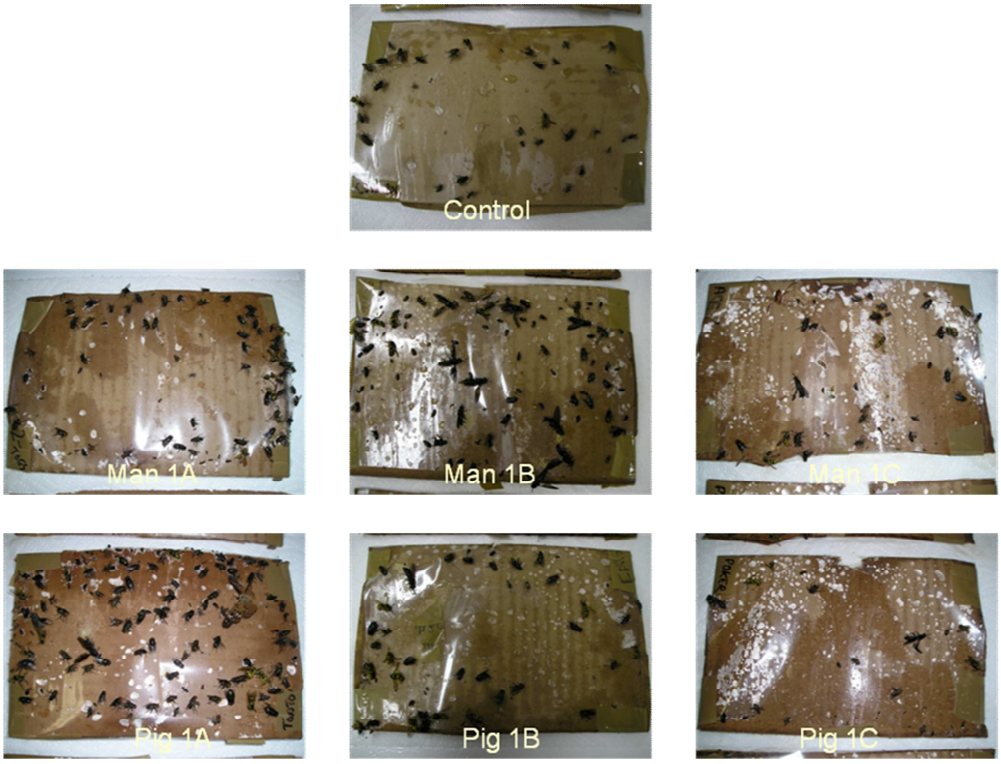


Figure 5.4.3.5 Sticky traps (Day 10-11) from Experiment 1

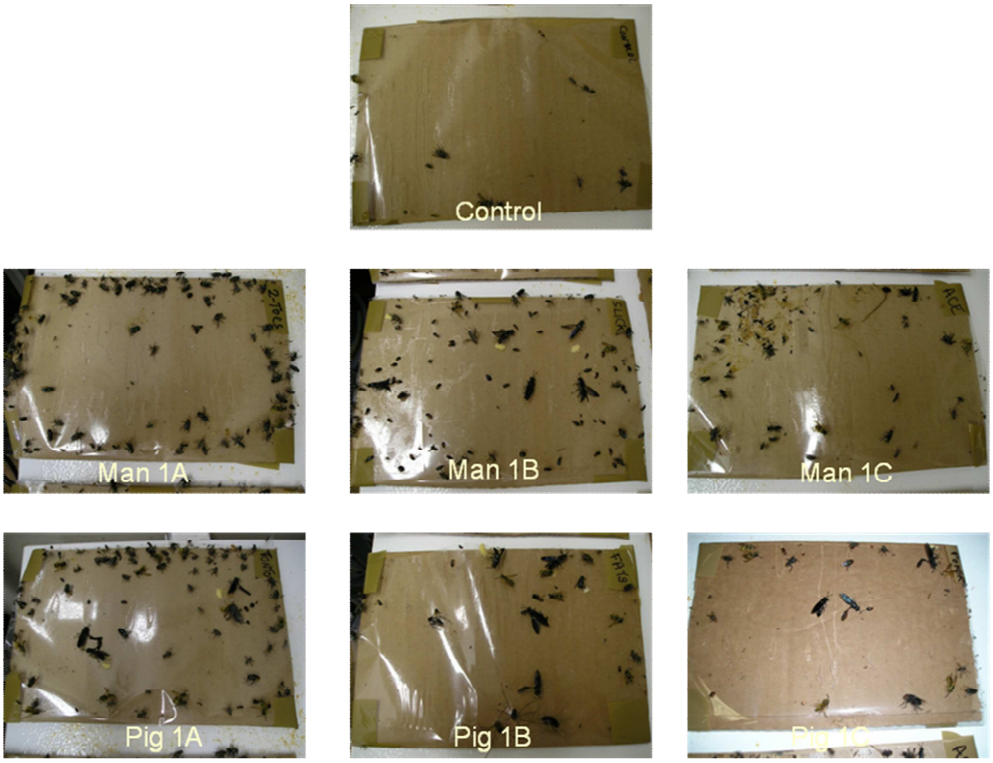


Figure 5.4.3.6 Sticky traps (Day 12-13) from Experiment 1

Figure 5.4.3.7 Sticky traps (Day 14-15) from Experiment 1

Appendix II Abstracts of oral presentations

Study of decomposition rates and egg viability in a cold climate

Amoret P. Brandt

2nd Annual Meeting of the EAFE, London, UK (2004)

The estimation of PMI in countries with fluctuating temperatures, both seasonally and daily, is considerably more complicated than in those with fairly constant temperatures. Studies have therefore been carried out in Central London, at The Natural History Museum, to study the effect of cold winter temperatures on the decomposition rate of young piglets. Comparisons have been made between those laid outside on a roof, and those laid inside in an unheated, though warmer office, with limited access for insects. As a result of these studies, the most interesting aspect which arose was the viability of eggs. Although laid at the same time on both the inside and outside carcasses, those outside failed to hatch due to the cold temperatures, therefore limiting the decomposition rate considerably, whereas those inside hatched as expected. However, once the weather warmed up, the eggs laid outside hatched and the larvae continued their normal development. Laboratory studies were therefore carried out to assess how long the eggs of *Calliphora vicina* can remain viable in cold temperatures, and whether they are able to hatch into larvae once the temperatures rise, either due to improved weather conditions, or the removal of the body inside.

Study of decomposition rates of pig carcasses and viability of *Calliphora vicina* in the UK climate

A. P. Brandt

XXII International Congress of Entomology, Brisbane, Australia (2004)

The estimation of post mortem interval (PMI) in a country such as the UK which experiences fluctuating temperatures, both seasonally and daily, is considerably more complicated than in those countries with fairly constant temperatures. In order to assess the effects of the different seasons on decomposition rates and insect species composition, piglet carcasses were put out at The Natural History Museum in Central London. Significant differences were recorded from carcasses put out at different times of the year, and between those laid outside on a roof, and those inside the building, where insects had limited access to the carcasses. Although oviposition by the primary invaders occurred at the same time on both the indoor and outdoor carcasses, the rate of development of *Calliphora vicina* on the outdoor carcasses was significantly slowed down due to the cold temperatures experienced, thus limiting the decomposition rate considerably. Further laboratory based studies were carried out to assess the effect of cold temperatures on the different life stages of *Calliphora vicina*.

Variation of insect succession on cadavers: the effect of reduced accessibility

Ian R. Dadour & Amoret P. Brandt

3rd Annual Meeting of the EAFE, Lausanne, Switzerland (2005)

Homicides and suicides continue to occur where corpses are left to decompose in places where insects have reduced accessibility. The fundamental principles of forensic entomology are typically the succession of insects and other arthropods on decomposing bodies. Succession follows a prescribed sequence, whereby different species inhabiting the same ecological niche are attracted to the corpse at a certain stage of decomposition. So what happens when the decomposing body is made less accessible to the insects? Is the composition of the insect fauna and the sequence they visit the corpse disrupted? What if the usual primary invaders, the Calliphoridae, are unable to access the body in the initial stages of decomposition and will this result in a miscalculation of the post-mortem interval?

Two particular studies are used to illustrate this problem. Firstly, a series of experiments carried out in Western Australia where pigs have been killed with CO and decomposed in semi-sealed cars. Secondly, a human cadaver was left to decompose in a standard household plastic wheelie bin in Knoxville, Tennessee. In both studies, a comparison with control cadavers ie. pigs/human (not concealed), revealed significant disruption to the expected faunal succession.

The effect of larval mass on development rates of fly larvae feeding on human cadavers in Knoxville, Tennessee, and dispersal of post-feeding larvae

Amoret P. Brandt & Ian R. Dadour

3rd Annual Meeting of the EAFE, Lausanne, Switzerland (2005)
17th Meeting of the IAFS, Hong Kong, China (2005)

In July 2004, the decomposition of a human cadaver was studied over a 2-week period. Twice a day, samples of the insects invading the body were collected, mainly Diptera larvae, but also Coleoptera, Hymenoptera and adult Diptera. The internal temperatures of the body (buccal, rectal and within the torso), together with the ambient temperature were recorded every 15 minutes by dataloggers using electronic sensor probes. Additional temperature readings were taken of the larval masses using a handheld infrared thermometer.

Mean larval lengths of each sample collected (~100 samples) were mapped against the larval mass temperatures from where they were taken, in order to try and ascertain to what degree the increased temperature of the larval masses affected the larval development rate. At present, most practicing forensic entomologists take larval masses into account when calculating the age of the collected larvae, but the calculations used tend to be instinctive and anecdotal, rather than based on published data. It is hoped that this study may give an indication of to what degree larval developmental rate is affected by larval mass temperature.

In addition, the dispersal of post-feeding larvae was observed from four different cadavers, with respect to time of day, number of days, direction, distance, speed and numbers. Despite certain commonly held assumptions, such as "larvae move up to 20ft/6m away" and "larvae always move downhill", considerable variation in larval behaviour was observed between the four cadavers.

The use of thermal imaging to study the effect of larval masses on the development of blowfly larvae

Martin J.R. Hall & Amoret P. Brandt

4th Annual Meeting of the EAFE, Bari, Italy (2006)

Blowflies of the family Calliphoridae are the most significant group of arthropods among the carrion community in terms of their role in decomposition. An adaptation to feeding of blowfly larvae is their concerted activity in larval masses, also called “maggot masses”. These are found from the late second instar stage and are advantageous to the individual because: (i) they enable a communal action of regurgitated enzymes to more rapidly break down carrion tissues; (ii) the concerted metabolic activity raises larval mass temperature, thereby giving potential for accelerated development or for development at ambient temperatures below those normally considered suitable. The latter effect could enable the blowfly population to extend its colonisation of carrion into the winter period. In the field, larval masses have been observed to reach 20-28ºC above ambient, but how to account for larval mass effects on larval development remains one of the most enigmatic problems within forensic entomology. At present, there is no standard protocol for analysing larval mass effects, nor even for applying some correction factor in trying to determine rates of development in situations where they occur.

Most techniques for temperature measurement are invasive and the physical insertion of thermometers into a larval mass can swiftly result in a dispersal of the mass. This problem was avoided in the studies reported here by the innovative use of a non-invasive technique, a thermal imaging camera. Although thermal imaging is commonly used in medicine and has even been used in forensic medicine, this technique has never been used before to study larval masses on cadavers. The equipment used was a FLIR Systems AGEMA 570 infrared camera and the images captured were analysed using FLIR Systems ThermaCAM Researcher Professional 2.8 SR-1 software. A combination of standard and close-up lenses enabled the surface temperature of larval masses and of individual larvae to be measured. The thermal dynamics of larval feeding on three still-born piglets, experimentally infested with *Calliphora vicina* (Diptera: Calliphoridae), were recorded, from oviposition to larval exodus. The experiments took place within an unheated room during the UK winter, when conditions for larval development were marginal. To complement the studies on carrion, larvae were reared on liver at different densities to study the relationship between larval density, larval age and the heat generated by a larval mass. In addition, studies were undertaken of the rate of heat loss by larvae of different densities, moved from a warm to a cold environment, to demonstrate the thermal benefits of feeding in a larval mass.

"The Body Farm "

Ian R. Dadour & Amoret P. Brandt

4th Annual Meeting of the EAFE, Bari, Italy (2006)

The Anthropological Research Facility (ARF), aka “The Body Farm”, was established in May 1981 by Dr William Bass, as a simple chainlink enclosure with a single donated body inside. Twenty-five years on, the Facility is now a 4-acre site and part of the Forensic Anthropology Center, University of Tennessee, Knoxville. They receive 30-50 body donations a year, with the remains eventually accessioned into the William M. Bass Donated Skeletal Collection, the largest collection of modern human bones in the world.

The main focus of ARF has been to build up the modern bone collection, which has led to other scientists being invited to the Facility to observe and understand the processes of postmortem decay. The primary aim for these projects is to improve the determination of post-mortem interval and to aid in cases where bodies are not easily identifiable. In addition to this, information on each donor is added to “ForDisc” (short for "Forensic Discrimination") a database in which all the skeletal measurements used for identification are logged, most importantly sex, race, age and stature. In recent years the Facility has also played an increasingly important role in developing or testing new forensic technologies, and also in training law-enforcement groups like FBI agents, crime-scene technicians, and cadaver dogs and their handlers.

Only a handful of Forensic Entomologists have had the opportunity to carry out research at the Facility, and in July 2004 we were able to study the decomposition of a human cadaver over a 2-week period. Twice a day, samples of the insects invading the body were collected, mainly Diptera larvae, but also Coleoptera, Hymenoptera and adult Diptera. The internal temperatures of the body (buccal, rectal and within the torso), together with the ambient temperature, were recorded every 15 minutes using electronic dataloggers. Additional non-invasive temperature readings were taken of the larval masses using a handheld infrared thermometer.

In addition, the dispersal of post-feeding larvae was observed from four different cadavers. Despite some commonly held assumptions, considerable variation in larval behaviour was observed between the four cadavers, a factor which should be considered when searching crime scenes for the oldest larvae, which may be post-feeding and therefore have moved away from the body to pupariate.

During the same two week period, a second body was monitored as it decomposed in a standard plastic "wheelie bin ". Infestion of this body was much slower, due to the relative inaccessibility of the body to blowflies. The usual faunal succession was severely disrupted, which could have profound implications for the calculation of PMI on bodies which have been wrapped, buried, submerged in water or otherwise made less accessible to necrophilous and other associated insects. Eight months later this cadaver was revisited when it was removed from the bin and buried in a shallow grave during the FBI’s annual "Body Recovery School", and a year following that it was exhumed and re-examined for insect evidence.

The use of thermal imaging to study larval masses on piglet and human cadavers

Amoret P. Whitaker

Natural History Museum Postgraduate Research Day (2007)

The first insects attracted to a fresh cadaver are usually the blowflies (Diptera: Calliphoridae), which lay their eggs in the orifices of the body and other protected regions, such as the folds of the skin or clothes, and under the body itself. The eggs hatch out into 1st instar larvae, which feed on the soft tissues of the body, growing rapidly through the 2nd and 3rd instars. By the late 2nd instar and throughout the 3rd instar they congregate together, forming larval (or maggot) masses. The temperature within the larval mass is often significantly higher than the ambient temperature, sometimes 20°C+, due to the cumulative metabolic activity of many larvae feeding in close proximity. Because the rate of development of larvae is dependent upon temperature, this increased temperature could result in an accelerated rate of development, but to what degree has not been determined.

The temperature within larval masses is often measured using a temperature probe, but not only does this cause the mass to disperse, it only measures the temperature within a very small area of the mass. In this novel study, we used a thermal imaging camera to record and measure the entire larval masses which formed in small piglet cadavers, throughout the period of decomposition. Four months later, a three week field trip to the Anthropological Research Facility in Knoxville, Tennessee enabled the same thermal imaging method to be carried out on larval masses on human cadavers, from fresh through to skeletonisation/mummification. Initial analysis suggests that the larval masses retain a consistently elevated temperature over a significant period of time, despite the movement of the larvae and the decomposition of the cadaver.

The use of thermal imaging to study larval masses on human cadavers

Amoret P. Whitaker (2007)

5th Annual Meeting of the EAFE, Brussels, Belgium (2007)

The most forensically useful group of insects are the blowflies (Diptera: Calliphoridae), as these are the first to colonise a fresh cadaver. Their rate of development is dependent on the temperature under which they develop, and they can therefore be used to estimate minimum post mortem interval. However, in the late 2nd and throughout the 3rd instar stage, the larvae feed in aggregated groups, called larval masses. The core temperature of these masses is usually elevated above the ambient temperature, such as in studies undertaken in Knoxville, USA on human cadavers, with temperatures of 42°C being recorded in larval masses, 20°C above the ambient temperature. Therefore for some period of time during their development, the larvae are being exposed to temperatures higher than that of ambient, although so far the effect of elevated larval mass temperatures on larval development has not been quantified.

At EAFE 2006 in Bari, Hall & Brandt (née Whitaker) presented a novel technique in which a thermal imaging camera (FLIR Systems AGEMA 570) was used to study the thermal dynamics of *Calliphora vicina* (Diptera: Calliphoridae) larvae. Three still-born piglets were manually infested with *C. vicina* eggs, and the development of the larvae, and dynamics of the larval masses, were recorded. Additional studies were undertaken of the rate of heat loss by larvae of different densities, moved from a warm to a cold environment, to demonstrate the thermal benefits of feeding in a larval mass.

A three week research trip to the Anthropological Research Facility at the University of Tennessee, Knoxville, USA, in July 2006 (Hart & Whitaker) enabled further thermal imaging to be carried out (using FLIR Systems ThermaCAM™ P65) on the larval masses present on human cadavers. In this study the larval numbers and the larval masses were much greater, due to the greater body mass of the cadavers, the increased ambient temperatures and the increased number of flies. Three cadavers were filmed for 10 days: two cadavers from 7 days

after they were placed in the research facility and colonised by blowflies, and a third cadaver was filmed from 1 day after it was placed outside until the larvae had dispersed 10 days later. Visits to the Facility were made twice a day, morning and afternoon, every day for 10 days. 50+ larval masses were recorded using time lapse photography, with up to 90 photographs being taken over a period of 9 minutes, or 1 photograph every 6 seconds. The core temperature of most of the larval masses retained a heat of up to 42ºC, with decreasing temperatures around the periphery. Samples of larvae were also taken from each larval mass, and the stage and size of these will be compared to the size and temperature of the larval mass from which they were collected to determine if there is a correlation which could be applied to estimations of post mortem interval.

Assessment of the validity of using pigs as a model for humans in forensic entomology studies

Amoret P. Whitaker & Martin J. R. Hall

7th Annual Meeting of the EAFE, Uppsala, Sweden (2009)
7Th Annual Meeting of NAFEA, Miami Beach, Florida, USA (2009)

Forensic entomology studies have been carried out by researchers on a wide variety of animal species, but the most widely used animal model is the domestic pig (*Sus scrofa domestica*), for economical, ethical and physiological reasons. However, it is likely that in the future, courts may question the validity of scientific research carried out on porcine rather than human cadavers, especially where post-mortem interval estimations are being used in criminal cases. Therefore it is important that the use of pig cadavers as a model for human cadavers is validated. One such study was carried out in 1989 (Schoenly *et al*., 2007) at the Anthropological Research Facility in Tennessee, using 3 pig cadavers and 1 human cadaver. Although the composition and succession of insects was found to be comparable between the two types of cadaver, no data was collected regarding Calliphorid larval development, therefore no conclusions could be drawn as to whether pigs are a suitable model for humans in estimating PMI.

In July 2008, a pig vs. human study was carried out at the Anthropological Research Facility in Knoxville, Tennessee. Three pig cadavers, each weighing 100lbs (45 kilos), and three adult male human cadavers were laid naked on the ground in three pig-human pairs. Each paired pig and human were placed 10m apart, and each pig-human pair were at least 50m apart. Tinytag dataloggers were used to record oral and rectal temperatures of each cadaver, and an infrared thermometer was used to record larval mass temperatures. Insect samples were collected twice daily, manually and using sticky traps and pitfall traps.

The results of this study will be presented here, including: location and rates of oviposition of Calliphorid flies; composition and succession of insects attracted to the cadavers; developmental rate of Diptera species, especially in the larval stages. Conclusions will be drawn as to whether the domestic pig is a good model for humans in estimating the minimum post-mortem interval.

Ref: Schoenly, Kenneth G., Haskell, Neal H., Hall, Robert D. and Gbur, J. Robert. (2007) Comparative Performance and Complementarity of Four Sampling Methods and Arthropod Preference Tests from Human and Porcine Remains at the Forensic Anthropology Center in Knoxville, Tennessee. *Journal of Medical Entomology* **44**(5): 881-894.

Comparison of larval masses on pig and human cadavers by IR thermography

Whitaker, A.P. & Hall, M.J.R. (2009)

7th Annual Meeting of the EAFE, Uppsala, Sweden (2009)

The evolution of larval masses on three pairs of human and pig cadavers was studied at Tennessee, USA, by infra-red thermography at twice-daily intervals over an eight day period in July 2009. Clear diurnal periodicity in the first two days of larval masses largely disappeared in the later stages as larvae matured and high temperatures were maintained until they left the masses. There were no significant differences between masses on pig or human. Differences between masses were associated with the dominant species, with *C. rufifacies* giving a lower maximum. IR thermography showed high temperatures of masses in the cadaver-soil interface, an area not measured by cadaver probes. While two-day resolution is not sufficient for a detailed temporal study, the technique shows promise for analysing and understanding the dynamics of larval mass development. Positive and negative aspects of the technique will be discussed.

Assessment of the validity of using pigs as a model for humans in forensic entomology studies

Amoret P. Whitaker & Martin J. R. Hall (2010)

Natural History Museum Student Conference, NHM, London (2010)
1st FIRN London and South East Student Regional Conference, University of Westminster, London (2010)
6th National FORREST Conference, Coventry University, UK (2010)

Forensic entomology studies have been carried out by researchers on a wide variety of animal species, but the most widely used animal model is the domestic pig (*Sus scrofa domestica*), for economical, ethical and physiological reasons. However, it is likely that in the future, courts may question the validity of scientific research carried out on porcine rather than human cadavers, especially where post-mortem interval estimations are being used in criminal cases, therefore it is important that the use of pig cadavers as a model for human cadavers is validated.

In July 2008, a study was carried out at the Anthropological Research Facility in Knoxville, Tennessee comparing insect succession and fly larvae development on three pig cadavers and three adult male human cadavers. Insects are temperature dependent, so monitoring the temperature at which they are developing is crucial in any Forensic Entomology study. Therefore three different methods were used for recording temperature: a) Tinytag dataloggers were used to record internal oral and rectal temperatures of each cadaver, b) an infrared thermometer was used to record external larval mass temperatures, and c) a thermal imaging camera was also used to record larval mass temperatures. Insect samples were collected twice daily, both manually and using sticky traps.

Initial results suggest that although there may be differences between individual cadavers, there are no significant differences between the insect activity on pig and human cadavers. The same composition of species and rate of development of fly larvae was recorded on both types of cadaver. In addition, the thermal imaging results showed no significant differences between larval masses on pig or human cadavers and in addition, also showed high temperatures of masses in the cadaver-soil interface, an area not measured by cadaver probes and often overlooked by forensic entomologists.

How long do empty puparia survive in woodland soil?

Amoret P. Whitaker (2010)

8th Annual Meeting of the EAFE, Murcia, Spain (2010)

In March 2002 Amanda "Milly" Dowler, a 13-year old schoolgirl, disappeared near to her home in Surrey, UK. Six months later her remains were found in woodland approximately 35km away in Hampshire. The body was skeletal, the skull found approximately 100m from the rest of the body, and no entomologist was called to the scene. Two years later, the police still had no leads and decided to call in forensic entomologists from the Natural History Museum, London, to search the scene for insect evidence to confirm whether the body had been moved to the site of deposition before or after active decomposition of the body. Very few empty puparia were found at the scene, so no conclusion was reached.

Did this mean that the body had not decomposed at the site where it was found? Or had the pupal cases disintegrated over time? How long do pupal cases remain intact in a rural environment? A search of the scientific literature revealed very little information on the subject, mainly reports from archaeological sites and a couple of case reports of exhumations, which prompted this study.

At a site in Hampshire woodland, 6,000 empty (ie. emerged) *Calliphora vicina* puparia were put out along a 50 metre transect – at each metre mark, 40 puparia were either buried to a depth of 5cm, left on the surface, or placed between the leaf litter and the soil. Over the next six years, the puparia were searched for and recovered, approximately one sample every month.

During the first two years, a significant proportion of puparia were recovered (70-90% at most sampling sites), but after about three and a half years, there were very few puparia found (only at 8% of sampling sites). During the first year or so, the recovered puparia were intact, whereas those recovered in the later stages were highly fragmented. A greater proportion of buried puparia were recovered, with the surface puparia being the least likely to be recovered. A number of factors could have affected the results. Firstly, there could be sampling error, i.e. it is possible that the puparia were still intact but simply not found. This is unlikely as the method used was consistent throughout the study. Secondly, the site was set in woodland of deciduous trees, so each autumn new leaf litter was added to the ground, making it harder to recover puparia on the surface and under the leaf litter in subsequent years. In addition there may be seasonal differences which affected sampling – in the summer the soil is relatively dry and falls away from the puparia, but in the winter the soil is wet and sticks to the puparia, making them harder to find.

This study suggests that the longevity of puparia depends on a number of factors, such as season and local vegetation, which should be taken into consideration when called upon to search for insect evidence, specifically blowfly puparia. The results also suggest that a significant proportion of pupal cases will still be found in woodland soil at least up to three and a half years after decomposition of a body.

Appendix III Posters presented

- 1st EAFE Conference, Frankfurt, Germany (2003)
- Postgraduate Poster Session, Natural History Museum, London, UK (2003)

Effect of Maggot Mass on the Development of *Calliphora vicina*

Amoret Brandt MSc DIC
Department of Entomology, The Natural History Museum, Cromwell Road, London, SW7 5BD, UK
School of Health and Life Sciences, King's College London, Franklin-Wilkins Building, 150 Stamford Street, London SE1 9NN, UK

Introduction

The size of maggots is often used as an indication of their age, and hence to determine the post mortem interval (PMI) in forensic cases. The occurrence of maggot mass, the tendency for maggots to congregate together, is generally accepted and it is assumed that this maggot mass results in an increase in temperature over the ambient temperature, thereby affecting the developmental rate of the larvae. Forensic entomologists often state that they take maggot mass into consideration when estimating the PMI.

However, few studies have been carried out to determine the effect of increasing the number of maggots, and hence the maggot mass size, on the development of the larvae. This study attempts to obtain quantitative data, by breeding a common European blowfly species, *Calliphora vicina* (Diptera: Calliphoridae), at different densities, up to a maximum of 1000 larvae in a maggot mass.

Materials and methods

Experiment 1

Experiment 1 was set up in order to determine:
•The number of larvae necessary to create a maggot mass large enough to generate a temperature above that of ambient.
•The ratio of grams of meat to number of larvae which is necessary to counteract any possible effects of competition.
•To what degree the above factors will affect the development of the larvae.
Three repetitions of each of 4 cultures were set up, using newly hatched *Calliphora vicina* larvae bred from wild stocks, thus giving varying ratios:
1) 20g/20 larvae 1:1
2) 20g/200 larvae 1:10
3) 200g/200 larvae 1:1
4) 100g/400 larvae 1:4
Larvae were fed on fresh liver, and sampled and measured at the third instar. Pupal measurements were also taken, and the pupal volume calculated (width x depth x length x π ÷ 6). The wing-vein dm-cu was used to measure adult size.

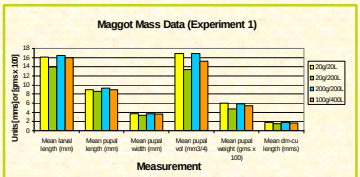
Experiment 2

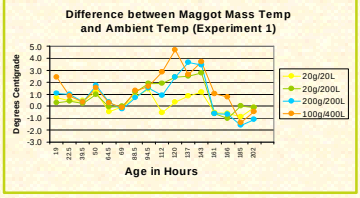
Experiment 2 was set up with the purpose of:
•Reducing the effects of competition.
•Increasing the number of larvae in each culture.
Two repetitions of each of 4 cultures were set up, using newly hatched *Calliphora vicina* larvae bred from wild stocks, thus giving varying ratios:
1) 20g/20 larvae 1:1
2) 100g/100 larvae 1:1
3) 250g/500 larvae 1:2
4) 250g/1000 larvae 1:4
In addition to the measurements taken in Experiment 1, larvae were sampled and measured daily from each culture.

Results

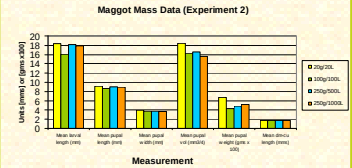
The results of Experiment 1 and Experiment 2 were not combined because:
•The methodology differed slightly (receptacles used/number of repetitions of each culture).
•Ambient room temperature differed between Experiment 1 (13.5°C-18.4°C) and Experiment 2 (21.1°C-23.9°C), affecting rate of development.
•Pupal measurements (length, width, weight) were carried out by two different people, therefore there may be variation.
The ADH (Accumulated Degree Hours) was calculated using the data from Experiment 2.

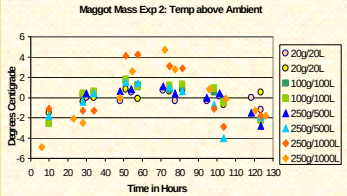
Experiment 1

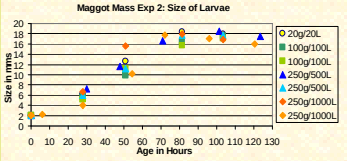




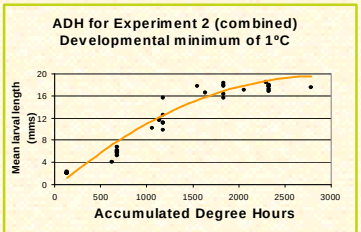
Experiment 2







ADH for Experiment 2



Discussion

Experiment 1

•Both cultures with a ratio of 1:1 (20g/20L & 100g/100L) exhibit the largest larval length, pupal measurements and adult dm-cu wing vein size.
•The culture with a ratio of 1:10, ie. that competing most intensely for the available food resource (20g/200L), exhibits the smallest larval length, pupal measurements and adult dm-cu wing vein size.
•The temperature within the maggot mass fluctuates from that of the ambient temperature for all cultures.
•The culture with the smallest number of larvae (20g/20L) remains closer to the ambient temperature than the other cultures.
•The culture with the greatest number of larvae (100g/400L) shows the highest temperature increase over the ambient temperature.

Experiment 2

•The measurements of larval length, pupal size and adult dm-cu wing vein size show a similar pattern to that in Experiment 1, although the differences between the cultures containing 100, 500 and 1000 larvae are not so apparent.
•The maggot mass temperature increase of the culture with the greatest number of larvae (250g/1000L) shows the highest temperature increase over the ambient temperature, and this is reflected in the slightly earlier development rate of the larvae.

ADH for Experiment 2

•Although carried out in a laboratory environment, the larvae in these experiments were not bred in incubators, and therefore the ambient temperature fluctuated during the experiments.
•Also, two of the of the seven cultures were reared during a slightly different time frame to the other cultures, and therefore the ambient temperature was not the same for all cultures during their development.

Conclusions

•An increase in maggot mass size results in an increase in maggot mass temperature, which may result in an increase in the rate of larval development.
•However, competition for the available food resources may result in reduced larval length, regardless of the size of maggot mass.
•The ADH calculation takes fluctuating ambient temperatures into account, which is essential when evaluating either real cases or experimental data in the field.
•Even within a laboratory environment, the ambient temperature may fluctuate, and therefore determining the ADH is vital to taking this into account when determining larval development rates.
•Further investigations will be carried out, with an increase in the size of maggot masses, more repetitions of cultures, and reduced competition.

Acknowledgements

Martin Hall: Of the NHM, for supervision, help and advice.
Bryan Turner: Of King's College, London, for PhD academic supervision.
Zoe Adams: Of the NHM, for upkeep of breeding stock and giving help and advice in the lab.
Claire Foggon: Of St. Hildas College, Oxford, for assistance with measurements (Experiment 1).

- 4th Annual Postgraduate Research Symposium, King's College London, UK (2004)
- 2nd EAFE Conference, London, UK (2004)
- Postgraduate Poster Session, Natural History Museum, London, UK (2004)

INSECT ACTIVITY ON PIG CARCASSES OVER WINTER IN LONDON

Amoret Brandt MSc DIC

Department of Entomology, The Natural History Museum, Cromwell Road, London, SW7 5BD, UK
School of Health and Life Sciences, King's College London, Franklin-Wilkins Building, 150 Stamford Street, London SE1 9NN, UK

Introduction

Forensic Entomology is the study of insects and other arthropods which live on carcasses, including human corpses. It is increasingly being used in many countries to help determine the Post Mortem Interval (PMI) of human remains, by studying the rate of development of the primary invaders, the blowflies (Calliphoridae), and also by monitoring the succession of arthropods on a cadaver, including the blowflies, parasitic insects and beetle larvae which eat the dessicated remains.

Aims

This exploratory study was carried out:

a) To compare the decomposition rates of indoor and outdoor piglet carcasses

b) To compare the insect activity between indoor and outdoor carcasses in winter

c) To compare the fluctuating ambient temperatures with the internal body temperatures of the pigs

Methods

Two young piglets were acquired from a pig farmer. The piglets had both died naturally within 24 hours of birth, and both weighed less than 2kg. One was placed inside a room at the Natural History Museum in South Kensington with a window left slightly open. The other was placed outside on the roof of the same building. Both piglets were at the same height off the ground, approximately 5 storeys. Ambient temperature and internal body temperatures were monitored using Tiny Tag detectors.

Indoor Pig

Set up

The indoor pig was placed in plastic tray inside a room, with a window left open a little to give access to flies. Two temperature probes were inserted inside the pig, one in the rectum and one in the mouth.

Day 5

The indoor pig is going through the bloating stage, caused by the activity of bacteria in the gut. A marbling effect can also be seen. Egg masses have been laid in the folds of the neck, in the ear and under the legs.

Day 11

The indoor pig is now full of maggot masses, at the rear end, in the abdomen, the neck and the snout. The internal temperature probes are secured to prevent them being dislodged by the movement of the maggots.

Day 15

The rear end and head of the body have been reduced to a liquid state by the action of 3rd instar larvae. 1st and 2nd instar maggots are also present, and egg masses have recently been laid around the neck area.

Day 27

The indoor pig is almost entirely desiccated, and the bones scattered by the movement of larvae. The 3rd instars have now turned into pupae, and these will be collected prior to hatching into adult flies. Smaller larvae are also present.

Outdoor Pig

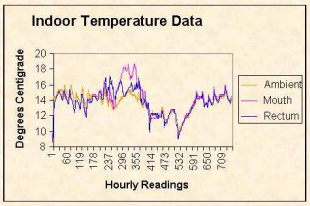
Set up

The outdoor pig was put in a cage (to prevent disturbance by rodents and birds), covered with plastic sheeting (to prevent too much rain getting in) and placed on an open roof. One temperature probe was inserted inside the rectum. The rainfall, wind and cloud cover was monitored on a daily basis.

Day 27

After 27 days, the outdoor pig is still almost entirely intact, apart from a single maggot mass at the dorsal rear end. It has only recently started to bloat and show a marbled effect. Of the few egg masses laid, just one hatched in situ, while others hatched only when taken into the lab.

Results and Conclusions



- The ambient indoor temp ranged from 10 ºC to 16ºC, and outdoor from -1ºC to 14ºC. By Day 27, the outdoor pig's body temperature had tracked that of the ambient temperature, while the internal body temperature of the indoor pig was 4ºC above the ambient temperature (see graph) at times when maggot masses were most active.
- Both pigs were visited by flies early on, and egg masses were laid on both carcasses. Initially only those on the indoor pig hatched into larvae, but was delayed on the outdoor pig until the weather improved. When egg masses were removed from the outside pig, they hatched within 24 hours in the lab, even having laid dormant in the cold for up to 8 days previously.
- The number of pupae taken from the indoor pig was 3988, weighing a total of 310g. The whole pig initially weighed 1.5kg, and 172.84g of skin and bones remained, therefore 88.48% of the pig was consumed by fly larvae.
- Even in low UK winter temperatures, flies are active and can lay eggs. However, the temperature must rise above a certain level before the eggs are viable and can hatch. This factor could drastically affect the calculation of PMI, suggesting a difference of perhaps weeks, therefore experiments on the viability of eggs at different temperatures will be carried out, in addition to indoor/outdoor studies of decomposition throughout the spring and summer.

Acknowledgements

Martin Hall: Of The Natural History Museum, for supervision, help and advice.
Bryan Turner: Of King's College London, for PhD academic supervision.
Zoe Adams: Of the Natural History Museum, for giving help and advice in the lab.
Ian Dadour: Of the University of Western Australia, for continual support and advice.

- 5th Annual Postgraduate Research Symposium, King's College London, UK (2005)
- 4th Annual Postgraduate Poster Session, Natural History Museum, London, UK (2005)

Effect of larval mass on development rates of fly larvae feeding on a human cadaver in Knoxville, Tennessee, and migration of post-feeding larvae

Amoret Brandt MSc DIC

Department of Entomology, The Natural History Museum, Cromwell Road, London, SW7 5BD, UK
School of Health and Life Sciences, King's College London, Franklin-Wilkins Building, 150 Stamford Street, London SE1 9NN, UK

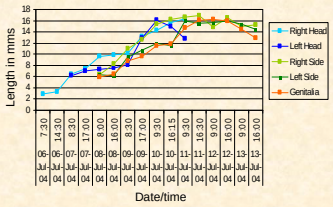
Introduction and Aims

This study was carried out over a two week period in July 2004 at the Anthropological Research Facility, Department of Anthropology, University of Tennessee, USA. The aim was to study larval masses on a human cadaver, their increased temperature and the effect on the developmental rate of the Diptera (fly) larvae feeding on the body.

Methods

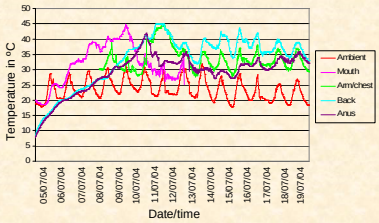
Datalogger probes were inserted into the mouth, anus and torso to record internal temperatures at 15 minute intervals, and another datalogger recorded ambient temperature and humidity. Twice a day, an infra-red thermometer was used to record the larval mass temperatures. At each visit the insects on the body were sampled. These included adult flies (Diptera) for species identification, adult and larval Coleoptera (beetles), Hymenoptera (wasps, ants) etc. Samples of fly larvae were collected from each larval mass for measurement.

Larval lengths



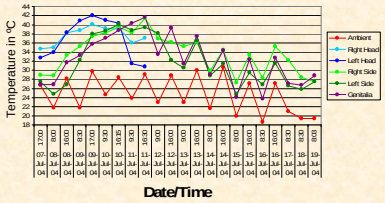
The larvae in the head region developed first, as the first eggs are laid in the facial orifices. Eggs were laid in the genital region and down the sides of the body on the third day, so the development of these larvae is two days behind those laid as eggs in the head region. All larvae grew to the same maximum length of 16mm before pupating.

Internal Body Temps



The ambient temperature fluctuated from 30ºC during the day down to 20ºC at night. The temperature in the mouth increased first, due to the first larval masses forming in this area. The torso and rectal temperatures were initially as low as 10ºC as the body had come straight out of the morgue. All temperatures rose no higher than 45ºC, above which the larvae would die.

Larval Mass Temps



Recording of the larval mass temperatures began two days after the egg masses were laid, as the larvae begin to mass during the late 2nd and the 3rd instar stages. The temperatures of the larval mass in the head became elevated first, where the first eggs were laid. The temperatures of the larval masses in the genital area and down the sides of the body follow, none reaching more than 42ºC.

Post-feeding larval (PFL) dispersal

On the sixth day after the first eggs were laid, the post-feeding larvae began to move off the body. The majority of them moved downhill, but the larvae from the head moved uphill. Larvae moved off the body on seven consecutive days, early in the morning. By each afternoon they had buried into the soil and under foliage to pupate.

Post-feeding larval dispersal was also studied on three other cadavers, and in each case variations were observed, leading to the following conclusions:

Assumptions	Exceptions
PFL disperse in many directions	PFL may follow a narrow trail
PFL usually move downhill	PFL may sometimes move uphill
PFL usually move 3-6m	PFL may move up to 15m
PFL usually stop and burrow under obstructions	PFL may move under, over or around obstructions
PFL usually leave a body in a single wave on one day	PFL may leave a body on many successive days

Conclusions

The first eggs were laid in the natural facial orifices of the body within a few hours, but it was 2 more days before eggs were laid in the genital region and along the sides of the body. In addition, the post-feeding larvae left the body on 7 successive days. Therefore, if the oldest larvae were not sampled, the calculation of PMI (Post Mortem Interval) could be underestimated by 9 days.

Both the internal body temperatures and the larval mass temperatures were substantially elevated above the ambient temperatures by as much as 20ºC, due to the metabolic activity of the feeding larvae, resulting in an increase in their rate of development. Forensic Entomologists often add a few degrees onto the recorded ambient temperatures to take this into account. However, in this study the larval mass temperatures rose at a steady rate despite the fluctuating ambient temperatures, a factor which requires further study.

Acknowledgements

Ian Dadour: The University of Western Australia, for organising the project.
Murray Marks, Bill Bass, Richard Jantz, Lee Meadows Jantz: Dept of Anthropology, University of Tennessee, for allowing the work to be done.
Martin Hall: The Natural History Museum, for supervision, help and advice.
Bryan Turner: King's College London, for PhD academic supervision.
The Anonymous Donors: without whom this work would not be possible.

➢ National Insect Week, Natural History Museum, London, UK (2008)

The Use of Thermal Imaging in Forensic Entomology Studies

Amoret Whitaker MSc DIC & Martin Hall DIC PhD

Department of Entomology, Natural History Museum, Cromwell Road, London, SW7 5BD, UK

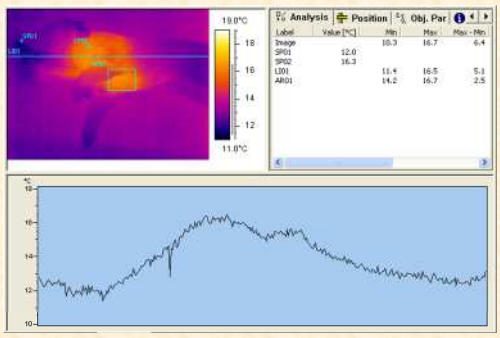
Introduction

Blowflies are usually the first insects to find a body after death and lay their eggs on it. These hatch into larvae and, as they grow, they go through 1st, 2nd and 3rd instar stages. Therefore by aging these insects when found on the body, the minimum post mortem interval can be estimated. Because the rate of development of the larvae is dependent on temperature, it is important to know at what temperature the larvae were developing.

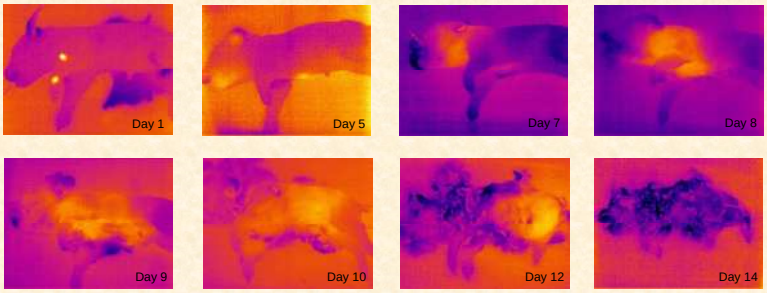
However, in the late 2nd and throughout the 3rd instar stage, the larvae form larval masses, and their combined metabolic activity results in an increased local temperature and therefore faster development. Even under very cold ambient conditions, the larval mass can stay active due to raised larval mass temperatures.

Current methods of measuring the temperature of larval masses are either invasive or only measure the temperature of a single spot at a single point in time. The use of thermal imaging allows non-invasive temperature measurements to be made, over an extended period of time and over a large surface area. In addition, the dynamics of larval masses are very not well understood and thermal imaging allows us to study them in more depth.

Analysis of thermal images



Images of stillborn piglet taken over 14 days



The above images follow the decomposition of a stillborn piglet. Colour indicates temperature, ranging from 11°C (purple) to 19°C (yellow), with a range of intermediate temperatures. The temperature scale is shown above right. The graph below shows the changing temperatures.

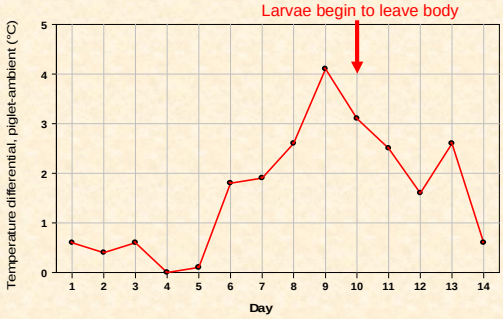
On Day 1, two adult blowflies (bright yellow spots) investigated the body, which was cooler than them. It took until Day 5 before small larval masses could be detected in the lower jaw and chest of the piglet. By Day 7, the larval mass was clearly visible in the throat of the piglet and throughout the following 7 days the larval mass moved through the body as the larvae fed on it, the mass becoming larger as the larvae themselves increased in size. By Day 12, the front end of the body was skeletonised and cooler than the substrate on which it was lying, and the larval mass was visible only in the rear end. By Day 14, all the larvae had left the body to pupate, leaving only skin and bones.

Camera set-up

FLIR Systems® AGEMA 570 camera

Graph of temperatures of piglet

Graph showing the increased temperatures of the larval mass within the piglet, above the ambient temperatures. The temperature is highest on Day 9.



Conclusions

- Thermal imaging can be used to record temperatures over a large area of a decomposing body and over a period of time
- It can be used to record temperatures of individual larvae
- It is non-invasive and therefore does not break up larval masses
- It can be used at crime scenes without contaminating the scene or disturbing the body
- It can be used to locate hidden larval masses
- The data can be recorded and presented as robust, scientific evidence

Acknowledgement

FLIR SYSTEMS

Appendix IV Publications

THE BARRISTER (Vol. 47)

Hart, A. J., Hall, M. J. R. H. and Whitaker, A. P. (2011)

The use of forensic entomology to assist the Criminal Justice System

Introduction

Entomology is the study of insects and other arthropods. Knowledge of entomology can be of great value in a forensic context, particularly in the estimation of timescales of the occurrence of an incident. This article explains its use in criminal investigations and how it may be of benefit to barristers, both prosecution and defence, when building their cases. A common application of forensic entomology is in the estimation of time since death (post-mortem interval or PMI): two to three days after death of a human, it can be difficult to estimate the PMI by standard pathological techniques. However, entomology can assist in determining minimum PMI both within and beyond these first few days and thus indicate a time frame of death which may help to implicate or exonerate a defendant. Research forms an important role in the development of this science as a forensic discipline and this is briefly outlined to provide background information. Forensic entomology is becoming a standard tool in criminal investigations. It is likely to become more frequently used as evidence in court and some of the questions that may be raised are addressed in this article.

How is forensic entomology used?

Insects and other arthropods can be used as evidence in criminal investigations particularly when a suspicious death has occurred. The estimation of the minimum time since death in such instances constitutes its main use but there are other potential applications of forensic entomology, some of which are listed below:

- Distribution of eggs or larvae (maggots) on a body may indicate presence of a wound from a gunshot or stabbing;
- Presence of insects foreign to the site of body discovery can suggest the body was moved from another location prior to deposition and potentially provide information as to the whereabouts of the original site;
- In cases of neglect or abuse, where a living person has become infested by insects (termed myiasis), ageing the insects can indicate the period of mistreatment;
- Toxicological analyses of insects that have fed on a drug user can indicate toxins present in the body when the remaining tissues are too degraded for analysis;
- Entomology may assist in cases of wildlife poaching and illegal importation of animal parts;
- Sourcing of illegal plant material, such as cannabis, by identifying the origin of any insects present;
- Health and safety instances of food contamination whereby insects indicate when and where the food may have been spoiled, either deliberately or through negligence.

The estimation of the PMI is where forensic entomology is most frequently applied. It requires an in depth knowledge of the insects that are most likely to be found on a body given the location and local conditions, as well as an understanding of their behaviour, physiology and ecology. It is vital, therefore, that a qualified forensic entomologist be used to collect and examine evidence.

What insects are forensically important?

A wide variety of insects can visit and colonise a body at different stages of decomposition and may include flies (Diptera), beetles (Coleoptera), bees, wasps and ants (Hymenoptera) and butterflies and moths (Lepidoptera). This relatively predictable order of insect visitors is called "succession". The most important of these in a forensic context are the blowflies (Calliphoridae). These are commonly known as

bluebottles and greenbottles and the adult flies have a striking shiny metallic-coloured appearance. Blowflies are generally the first insects to arrive at a dead body and in the greatest numbers and, therefore, they provide the most accurate means to estimate the minimum PMI.

Blowflies can be attracted to living bodies, but the odours emanating from a decomposing body attract significantly more, usually within hours of death, sometimes minutes, to feed on the soft tissues and decomposition fluids. Female blowflies lay their eggs on the body, usually in the natural orifices, but also in folds of clothing and along the interface between the body and the substrate on which it is lying. The eggs are the first stage in the life cycle of flies (see Fig. 1), and they hatch into tiny first instar larvae, one to two millimetres in length, before developing through into the second and third larval instars. In the late second, and throughout the third instar, larvae feed collectively, forming large 'maggot masses', the temperatures of which may be significantly higher than the ambient temperature. Once the third instar larvae have finished feeding, they generally move away from the body to find a suitable site to pupate, by which process they metamorphose into adult flies. Pupae are, therefore, usually found not on the body itself, but in the surrounding environment, such as buried in the earth or, if indoors, under carpets and furniture. They are small, approximately one cm long, ovoid, and reddish brown in colour. Once formed, the adult fly emerges from its pupal case (puparium) ready to feed, mate and, if female, seek out another body to colonise. The empty pupal case is left behind as evidence of fly emergence.

What research is being done on insects and decomposition?

Carrion feeding insects are highly efficient decomposers of dead bodies, whether animal or human, and without them decomposition would be significantly delayed. Five stages of decomposition have been described to assist the entomologist in assessing the remains at a crime scene: fresh, bloat, active decay, post-decay and skeletal. Each stage has a series of characteristic components relating the appearance of the body to its associated insect activity.

Generally, forensic entomology is studied using pigs as surrogate cadavers for humans; however, it is possible to study the decomposition of donated human cadavers at a number of facilities in the USA such as the Anthropological Research Facility ('The Body Farm'), University of Tennessee, Knoxville, USA. The advantage of working at such facilities is that the effect of different environments, climates and seasons on decomposition can be studied. Due to the climatic conditions at the Knoxville Facility, during the summer months almost the entire process of decomposition can be observed within two to three weeks, from fresh through to partial skeletonisation or mummification. Vital forensic entomology studies can be carried out by recording the internal and external temperatures of the body and the associated larval masses throughout the decomposition process, and by collecting, identifying and aging the insects. Technological advancements in research, such as the use of thermal imaging to enable more robust study of larval mass thermal dynamics, can assist the forensic entomologist in producing more accurate methods for determination of the minimum PMI.

What does a forensic entomologist do?

A forensic entomologist may be contacted by the police or forensic provider in the case of suspicious or untimely death to assist in the collection and preservation of insect evidence at the scene and/or the post-mortem. This is followed by the identification and analysis of the insect evidence to assist in the estimation of the minimum time since death.

There are three main advantages of using a qualified forensic entomologist:

1. Recognition of insect evidence: early stages of insects, such as eggs and first instar larvae, are small and inconspicuous and therefore easily missed. Both the egg and the pupal stages are sedentary and therefore not always recognised as being of insect origin. Mature third instar larvae may have left the body after completing feeding and could be overlooked, being widely dispersed some distance away. In addition, the absence of insects could also be important in the context of the case and would require evaluation by the expert.
2. Correct methodological protocols: insect evidence will be collected and preserved in the correct manner (see Fig. 2.), in accordance with guidelines set out by an international panel of experienced forensic entomologists, thus enabling proper analysis and accurate identification.

3. Production of robust scientific data: the evidence collected will be interpreted in a systematic and scientifically robust manner resulting in the production of a written statement or report which can be used for evidential purposes. The forensic entomologist is generally trained as an expert witness, and therefore is competent to appear in court if required to present their evidence in a manner that is clear and understandable. This is an important consideration given the potentially complex nature of the work.

Preservation of the insects recovered from a cadaver or scene of crime is relatively straightforward as long as the recommended guidelines are followed. Larvae are killed by placing them in near boiling water, which also maximally extends them and helps to prevent degradation. They are then placed in 80% ethanol which will preserve them almost indefinitely. Eggs, pupae and adult flies can be placed directly into 80% ethanol. Pupae should be carefully pricked with a pin before immersion. Live specimens of eggs, larvae and pupae should also be collected and reared to adulthood as this can greatly assist in their identification.

Temperature is the main factor influencing the rate of development of insects, with increased temperatures leading to an increase in the speed of development and *vice versa*. It is therefore essential to be able to estimate the temperatures under which the insects were developing at the scene. This can be done by placing an electronic datalogger at the scene for 7-10 days. Temperature data is obtained from the nearest weather station for the same period and also for the preceding period, since the last known sighting of the victim, i.e. for the potential period during which the insects were developing on the body. A regression analysis is carried out on the two sets of data and, using the resulting equation and the weather station data, the temperatures at the scene before the body was found can be estimated, i.e. those to which the insect evidence was exposed.

Having identified the entomological specimens, they are aged by determining their life stage, measuring the larval length or by dissecting the pupae. The time taken to reach this stage can then be estimated given the temperatures at which they developed, using published data on the development of that species. By this method an estimate of the minimum PMI can be given, actually the time when insects first laid eggs on the body, and be used by the police to aid their investigation. This may be for intelligence purposes in the early stages of the investigation to assist with the identification of a potential offender or as part of the evidential process after an individual has been arrested.

It is important to stress that any estimate of the PMI is the minimum time since insect eggs were laid on the deceased. The individual may have been dead for longer than the insect activity suggests if the body had been inaccessible to the first colonising insects, for instance if it was wrapped or contained in some way. Burial, immersion in water and the presence of clothing can significantly affect the ability of the insects to colonise a body.

How is the evidence presented in court?

The advantages of meetings or case conferences to discuss the entomological evidence prior to the commencement of a trial cannot be overemphasised as the evidence can be complex and highly detailed and needs to be presented in an understandable and clear manner but without compromising the interpretation and conclusions of the work. Pre-court meetings enable the forensic entomologist to explain any theoretical or practical questions that the barrister may have and to reduce the potential confusion of long-winded explanations in court. It is important that the prosecution and defence barristers have at least some understanding of the field as this will reduce the number of unnecessary and potentially confusing questions.

What questions may be asked in court?

The following list contains a few of the questions that may be raised in the court room in relation to forensic entomology. Obviously, there may be many more questions relating specifically to the case that arise.

1. *Can you explain what forensic entomology is?*

As it is important to use straightforward language and to minimise jargon, this article may be helpful. Some members of the jury will inevitably be interested in this relatively uncommon evidence type, while others may perhaps be uncomfortable with the idea of insects feeding on bodies, so a suitable balance will need to be found.

2. Can you explain how you obtain the time of death?

A forensic entomologist cannot determine the actual time of death, as this is outside of their field of expertise and is the role of the forensic pathologist. What a forensic entomologist can do is provide a *minimum* PMI by assessing the period of insect activity on the body. Remember, the deceased may have been dead longer than the estimate given by the forensic entomologist as the insects may not have had access to the body or located it until sometime after the actual time of death.

3. If the body was outside, how long would it take for flies to find?

It depends on the environment and season in which the body is found, but if the conditions are right, and blowflies are in the vicinity, they can alight upon a body within minutes of death and odours of decomposition can attract flies in increasing numbers over time, even from some distance away. The fly uses odour receptors in its antennae to respond to specific chemicals and will follow the increasingly strong odour trail upwind. It would be unusual for a body to be exposed outdoors for greater than 24 hours without there being some insect activity, particularly during the summer.

4. What insects might you find on a body?

Blowflies are generally the first insects to arrive at the body and in the greatest numbers. However, there may be different species of blowflies, depending on the location. For example, the bluebottle *Calliphora vicina* would be more likely to frequent urban locations, while the greenbottle *Lucilia caesar* prefers more rural, especially wooded, conditions. There then follows a succession of different insects which can include various groups of other flies and beetles as the body continues to decompose.

5. Will flies find a body at night?

Flies are generally inactive at night and will therefore be unlikely to lay eggs in darkness, a fact which also needs to be taken into account when estimating minimum PMI.

6. *When a blowfly lands on a body how soon do they lay eggs?*

Female flies will be attracted to a body to feed, because they need to ingest a protein meal in order for their eggs to develop. An already mated female may be ready to lay eggs almost immediately if a suitable substrate is present. Males may be attracted to a body in lesser numbers in order to try and mate with the females.

7. Where do flies lay eggs?

Female blowflies can lay eggs all over the body, but will initially deposit eggs in and around the orifices of the head before moving down to the genital region and other areas, including sites of wounding if present. The eyes, ears, nose and mouth provide a moist and protective environment, where eggs are less likely to desiccate or be predated, and also provide a food source for first instar larvae that is easier to digest than unbroken skin.

8. Does rainfall affect the insects' behaviour?

Flying insects will be less active, so heavy rainfall is likely to delay egg-laying by blowflies. Heavy rainfall may also affect decomposition by washing egg masses off the body and drowning larvae.

9. How long does it take for blowfly eggs to hatch?

This will depend on the temperature and species. A ball-park figure on a typical English summer's day is about 24 hours. The emerging first instar larva would then take about another 24 hours to reach the second instar stage and a further 48 hours to reach the third instar larval stage. Under colder conditions, such as in winter, this may take weeks.

10. ***How long would it take from the eggs being laid until they had completed the third stage larval stage?***

Following on from question 9, a third instar larva would take an additional three to four days to complete its development. Thus, total development for the egg and larval stages of this 'average' blowfly would be seven to eight days in summer conditions. The third instar larvae would finish feeding, evacuate their guts and leave the body, entering the 'wandering' or post-feeding dispersal stage.

11. ***What happens when there is a maggot mass?***

As the larvae feed and develop, they can congregate in large numbers, forming what is known as a 'maggot mass'. The temperature within these masses is often elevated well above the surrounding temperatures (e.g. 20°C above ambient), which will increase the rate of development of the larvae within the mass. This should be taken into account in the estimate of the minimum time since death, ideally by recording the temperature of the larval masses on the body. However, this is a complex issue because larvae feeding in a mass move within it and are not exposed to the highest temperatures continuously.

12. ***What makes the larvae wander?***

When the larvae have finished feeding, most leave the food source to find a suitable site for pupation, most likely to reduce the risks of predation and parasitism. These post-feeding or pre-pupal larvae will burrow into the soil or under rocks or vegetation if outdoors; if indoors, they may hide under furnishings, in cracks under the skirting board or beneath any carpet. Larvae of the black blowfly (*Protophormia terraenovae*) are an exception to this, and do not tend to move far from the body.

13. ***How far do they wander?***

In soft soil, larvae tend to burrow down quite close to the body, typically within one to three metres. On concrete, clay or hard earth they may move some distance away (ten to twenty metres of more) in order to locate a suitable site for pupariation. It is important to remember that because of this behaviour, the oldest insects (i.e. those which will give the most accurate minimum PMI estimate) may be found some distance away from the body.

14. ***How long do they remain as pupae?***

During a warm summer, the pupal stage would last about a week and then the fly would emerge to begin the whole cycle again In some cases, just the empty pupal cases may be found, but these can still be identified to species, and a minimum PMI given for one generation of blowflies. A pictorial representation of this life cycle could be provided for the jury if required.

15. ***What flies might you find at a crime scene?***

This would entirely depend on the location and the time of year that the body was deposited. For example, you would expect to find different flies in a woodland (e.g. *Lucilia caesar*) as opposed to grassland (e.g. *Lucilia sericata*). It can sometimes be a tight delimitation; a body found next to a hedge could potentially have a different population of flies to a body that was four metres away in grassland. Also, some flies are more seasonal than others, for example the bluebottle blowfly *Calliphora vomitoria*, is active from spring through to autumn, whereas its closely related sister species, *Calliphora vicina*, also a bluebottle, can be active throughout the year, although in lower numbers during winter.

16. ***How can you be sure that the insect evidence has come from the body and not from a dead animal?***

When collecting evidence from a crime scene, the forensic entomologist should normally dig a number of control sites, i.e. a distance of ten metres or so from the body, where one would not expect to find post-feeding larvae and pupae, which will indicate that the insect evidence close to the body has in fact originated from it.

17. ***How do you calculate the minimum PMI?***

This can be relatively complex, so a simple and brief summary of the theory can be provided unless more detail is required. It can include the relationship between temperature and insect development,

the necessity of recording temperatures at the scene and their comparison with temperatures from the nearest weather station, identification and ageing of specimens and the estimation of the time taken to reach that age in combination with published data.

18. ***Where do you obtain the development data from?***

There are an increasing number of peer-reviewed scientific papers on the development of certain species of flies and this information is used when estimating the minimum time since death. However, it should be noted that there is a lack of such data for some forensically important fly species, making it more difficult in some cases to determine an accurate PMI. Further research is being undertaken by scientists in an attempt to address this.

19. ***You say the victim must have been dead on a certain date, could they have died earlier than this?***

Yes, the estimate given is a *minimum*, and flies may have been prevented from immediately colonising the body, for example if the body was not accessible because it was in the boot of a car or tightly wrapped.

20. ***Can you not give me the actual time he died?***

A forensic entomologist will usually give a range of dates for the possible minimum time since death, due to the variability of the biological and climatic systems involved. It may be possible to give a narrower time frame in some cases but it depends on the entomological evidence collected at the scene. Generally, the longer the PMI estimate, the broader the range of dates.

21. ***This is not a very precise science is it?***

Forensic entomology is the study of living organisms and, therefore, is subject to biological variation. However, the analysis is based on peer-reviewed publications and should always include some estimate of confidence intervals to account for the variation.

22. ***Do you follow the same approach as other forensic entomologists and meet appropriate quality standards?***

The forensic entomologist should follow standard techniques, such as those detailed in Amendt *et al* (2007). These have been validated by an international board of forensic entomologists.

23. ***How would you present a case involving forensic entomology to the jury?***

This would follow the usual format for other forms of scientific evidence as detailed in a Criminal Justice Act statement. Visual aids such as an illustration of the life cycle of the blowfly and PowerPoint slides could be used to assist the jury understanding this specialist area. A case conference before the trial would enable clarification of key points which should be presented to the court.

Case example

In July 1999, the badly burnt body of a man was discovered by some children in an old ammunition bunker in Essex. Because of the extensive damage incurred by the body from the fire, which had apparently taken place in the room, the forensic pathologist had great difficulty in estimating a time of death in this case, which was eventually shown to be a suicide. There were large numbers of fly eggs and larvae on the body, so a forensic entomologist was called to the scene. There was no evidence of burnt insects on or around the body, so adult flies had laid eggs on the body after the burning had occurred. The temperature in the underground bunker was relatively constant at about 14°C. The larvae were measured and several distinct size cohorts were found, representing larvae that had hatched from eggs laid on several consecutive days. The largest and, therefore, oldest larvae were estimated to have been laid as eggs six days before the body was found, establishing a minimum PMI. That information enabled the police to focus their investigation to a particular time frame and subsequent witnesses recorded that a fire had been seen in the bunker on the night before the flies were likely to have found the body.

Conclusion

This article provides information on forensic entomology for barristers and the wider Criminal Justice System and attempts to answer just a few of the questions that may arise in court when such a case is presented. The application of forensic entomology is becoming more prevalent in the UK and it is now an accepted tool for use by criminal investigators. In the majority of cases, forensic entomology reports are used to give a time period in which the investigation should be focused. Only in cases where time of death is a critical factor or in contention will the forensic entomologist usually be required to attend court as an expert witness. Therefore, it may still be rare for a member of the legal profession to have to deal with this evidence type. However, when such cases arise, it is imperative that the barrister has at least a rudimentary knowledge of this specialised field.

The Use of Forensic Entomology in Criminal Investigations: How it can be of benefit to SIOs

Dr Andrew J. Hart
The Forensic Science Service, London

Amoret P., Whitaker MSc DIC and Dr Martin J. R. Hall
The Natural History Museum, London

Abstract

This article introduces the science of forensic entomology, its application in criminal investigations and how it may be of use to Senior Investigating Officers (SIOs). Its use in the estimation of the minimum time since death or post-mortem interval is described, which is often one of the key questions asked by a SIO during a murder investigation. After 48 to 72 hours of death occurring, it can be difficult to obtain such an estimate by traditional pathologists' methods and the time interval beyond this is where entomology is potentially of great benefit to the police. Research on human donor bodies is also discussed, which forms a backdrop to the use of forensic entomology in casework.

Contents

All correspondence should be addressed to: Dr Andrew J. Hart, Violent Crime, The Forensic Science Service, 109 Lambeth Road, London SE1 7LP
Email: Andrew.Hart@fss.pnn.police.uk

1 Introduction

Forensic entomology is the study of insects and other arthropods in relation to the law, most commonly to assist in criminal investigations by the interpretation of insect evidence in cases of suspicious death (Amendt *et al*., 2007; Anderson, 2001; Byrd and Castner, 2001; Catts and Haskell, 1990; Greenberg, 1991; Smith, 1986). While this is one of its predominant uses, entomological techniques can also be applied in a variety of other ways which do not necessarily always include a criminal aspect, such as in civil cases or in a medical context. The scope of forensic entomology is therefore very broad and is sometimes called 'medico-legal entomology', particularly in the USA and Canada. Some of its applications include the following examples:

- An infestation of insects on a living person, often the young or the old, is called myiasis, and indicates a case of neglect and/or abuse, even if discovered after death.
- If the victim was taking drugs, toxicological analyses of any insects which have fed on the body can reveal the presence of such toxins, which is especially important if the body itself has decomposed beyond examination.
- In cases of importation of illegal plant material such as cannabis, any insects present on the plants can potentially identify where it was grown.
- In cases of wildlife poaching, insect evidence may aid the investigation.
- Entomological material that has contaminated food may provide information as to how long the food has been spoiled for health and safety investigations. Insects may also be deliberately placed into food substances for blackmail or fraudulent reasons.
- During investigation of insurance fraud where for example individuals falsely claim to have structural damage caused by termites;
- Endangered species of insects may be illegally imported and traded by collectors.

However, the main use of forensic entomology is for the estimation of the minimum time since death or post-mortem interval (PMI) to aid murder investigators (Anderson, 2001; Dadour *et al.,* 2001; Goff, 1993), which requires an understanding of the taxonomy, biology and ecology of the insects involved.

2 Forensically Important Insects

The decomposing body attracts a succession of insects in a relatively predictable order. These may include flies (Diptera), beetles (Coleoptera), butterflies and moths (Lepidoptera), and wasps, bees and ants (Hymenoptera) among other groups (Turner, 1987). Flies and beetles are the most frequent visitors, as both immature insects (larvae) and adults. However, the bluebottles and greenbottles (blowflies or Calliphoridae) are generally the most forensically important insects (Greenberg, 1991) because they are the most numerous insects on dead bodies and they are usually the first to arrive. Therefore they are the most useful group of insects for estimating the minimum time since death.

As the body begins to break down, flies are attracted within minutes to the odours emanating from the decomposing tissues and fluids. Upon arrival they may begin feeding and some of the females lay eggs in and around the natural orifices of the body (eyes, ears, nose, mouth and genital region), although eggs may also be found in the hair, between the body and the ground, in the folds of clothes, or in open wounds. In the latter case, the presence of eggs or larvae may indicate the presence of an injury caused by a knife or a gunshot.

The eggs hatch out into tiny first instar larvae (commonly called maggots) and pass through two more instars. In the late second and throughout the third stage the larvae form feeding ‘maggot masses’, which can lead to an increase in temperature of the mass, sometimes as much as 20ºC above the surrounding temperature, depending on the numbers of larvae. When the larvae have finished feeding, they disperse away from the body and, depending on the nature of the ground, may burrow into the soil, or move

under stones, logs and vegetation. The larvae metamorphose into puparia, which are immobile while the adult fly develops inside before eventually emerging and beginning the life cycle again.

3 Stages of Decomposition and Associated Insect Activity

Insect activity has a major role to play in the decomposition of a cadaver. Payne (1965) found that carcasses of piglets exposed to insect activity lost 90% of their body weight in six days, whereas carcasses which were free of insects lost just 30% during the same period. Although decomposition is a continuum, its process has been described in five stages, each of which attracts different groups of insects, referred to as succession (Goff, 1993):

1. ***Fresh*** - blowflies are attracted to the body to feed and lay eggs.
2. ***Bloat*** - gases are released from the rapidly reproducing anaerobic bacteria in the body. The metabolic activity of feeding larvae can cause a localised increase in the body temperature. If the body is found on soil, fluid leakage combined with the formation of ammonia makes the substrate alkaline and repels the usual soil-living invertebrates.
3. ***Decay*** – openings created by the feeding activity of the larval masses allow the gases to escape, resulting in deflation of the body. By this stage adult and immature beetles are also present, feeding on the tissues of the body and predating upon the fly larvae, early colonisers of which have started leaving the body to pupate.
4. ***Post decay*** - most of the soft tissues of the body have now been consumed, leaving only mummified skin, cartilage and bones which may be fed on by beetles.
5. ***Skeletal*** - all that remains of the body are the bones and hair, with few or no carrion insects present.

The latter two stages were combined by Rodriguez and Bass (1983) and termed the ‘dry stage’.

The majority of forensic entomology research has used pigs as models for humans, for physiological, economical and ethical reasons, and extensive literature exists on this. However, to date there is only one location in the world where it is possible to study the decomposition process in donated

human cadavers, namely the Anthropological Research Facility, University of Tennessee, Knoxville, USA, known colloquially as 'The Body Farm'.

4 Research on Insect Activity and Decomposition of Human Cadavers

The Anthropological Research Facility was founded in the early 1980's by Professor William Bass, a pioneer of forensic anthropology, and is now managed by the Forensic Anthropology Centre based at the Department of Anthropology at the University of Tennesse, Knoxville (Marks, 1995). Bodies are donated to the facility where they are decomposed in a natural outdoor setting, enabling research to be carried out in areas such as entomology, soil ecology, microbiology, chemical ecology and histology. In addition, some of the bodies may be placed in various 'crime scene' scenarios, for example submerged in water, in a car boot, buried underground or placed indoors. Once the body has decomposed to the skeletal stage, the remains are collected, processed and then accessioned in the department's William M. Bass Donated Skeletal Collection to be used for future research, teaching and reference material in forensic casework. Details of the donors' gender, race, age, stature, life style and medical history, gathered prior to donation, are kept on record which greatly enhances the value of the collection. A facial photograph of the donor, taken while still alive, is also archived to assist with cranio-facial reconstructions and human identification studies. The facility is also used as a training ground for the FBI and international disaster body recovery teams.

There has been a paucity of research in the area of insect activity and human decomposition (Rodriguez and Bass, 1983; 1985) due to ethical constraints, and since its inception only a few forensic entomologists have had the opportunity to work at the Anthropological Research Facility. More recently, scientists from three institutions have been collaborating on a 4 to 5 year study at the facility into the role of insect activity on the decomposition of human cadavers: the Forensic Science Service, London, the Natural History Museum, London, and the University of Western Australia, Perth.

During the course of this study, a number of cadavers were laid out at the facility. Probes were inserted into the mouths, rectums and backs of the bodies in order to record the internal body temperature as decomposition progressed and ambient temperatures were also recorded for comparison. The temperatures of the larval masses that formed as they fed on the bodies were recorded using a non-invasive infra-red thermometer. Using these techniques it was possible to record the elevated temperatures that occur due to the aggregated feeding activity of the larvae; temperatures of up to 46°C were reached, about 20°C above the ambient temperature. Samples of insects that were present on and around the body were collected twice a day, killed by immersion in freshly boiled water, and preserved in ethanol. These have been retained for identification, measurement and analysis, with a view to detailing the development of these insects on the bodies in relation to the temperature. A proportion of the collected insect specimens were kept alive and reared through to adulthood in order to assist in the identification of the insects.

Photographic and written records were kept, cataloguing the process of decomposition for each of the bodies for the duration of research at the facility. Given the high humidity and temperatures recorded in Knoxville at the time of year when the studies were conducted, it was possible to observe and record decomposition from fresh through to part skeletonisation and mummification within a short time. There was a large amount of insect activity during this time, with thousands of larvae forming huge feeding masses on the body. Time lapse photographic images were taken to demonstrate this progression and to show the rapid development of such masses. Video recordings were also made to provide a record of the behaviour of insects and the changes in their activity throughout the decomposition process of human cadavers. In addition, thermal imaging was used to non-invasively record temperatures, which will give a greater understanding of the dynamics of larval masses, and how this affects the development of the larvae. Crime scene investigators only witness the process of decomposition at the point in time when a body is found, and

researchers generally work only with non-human carrion. Therefore, it would be useful in the future for such images to be used in the education of personnel involved in crime scene work and body recovery.

5 Role of the Forensic Entomologist in a Criminal Investigation

Ideally, when the victim of a suspicious death has been found the SIO should request that a forensic entomologist attend the crime scene and the autopsy to record and collect any insect evidence that may be present. This is for three main reasons:

1. A forensic entomologist is the most qualified person to identify insect evidence: eggs and early stage larvae are small, and may be hidden or otherwise inconspicuous and therefore easily overlooked. In addition, the oldest larvae may have already finished feeding and left the body to pupate, and therefore their presence may not be obvious. Even if there is no apparent insect evidence present, a forensic entomologist may be required to assess the possible reasons for this absence.
2. A forensic entomologist can be sure that the evidence has been collected, killed and preserved using the approved methods.
3. A forensic entomologist will make a systematic written and photographic record of the insect evidence collected in the context of the crime scene, followed by a detailed analysis, resulting in a written report and, if necessary, a court appearance.

When preserving insect evidence, blowfly adults, eggs and pupae can be put directly into 70 to 80% ethanol (alcohol), the latter being pricked first. Larvae should be immersed in freshly boiled water to maximally extend them and prevent bacterial decay, before placing them in ethanol. Preserved specimens are taken back to the laboratory where the species, lifestage and age can be determined. In addition, live specimens of eggs, larvae and pupae should also be collected (ensuring they are aerated and that feeding larvae have food), and reared through to adults to confirm identification of preserved specimens.

The rate of development of insects is largely dependent upon temperature: the hotter it is, the faster they develop, and conversely the colder it is, the slower they develop. (Donovan *et al.*, 2006). Therefore, it is essential that the temperatures at which the insects have developed are estimated. A forensic entomologist places an electronic datalogger at the crime scene to record ambient temperatures for 7 to 14 days. Temperature data for the same period is obtained from the nearest meteorological station to the crime scene, and that for the preceding period during which the body may have been there. A regression analysis is carried out on the temperatures from the two locations for the same time period, and based on this, the temperatures to which the developing insects were exposed before the body was found can be estimated. Once the fly species have been identified, the age of the preserved specimens can be determined by measuring the length of the larvae or dissecting the pupae, and comparing their stage of development with published data in the context of the temperatures at which they developed. Therefore an estimate of the post-mortem interval is produced which is then passed to the SIO either for intelligence or evidential purposes. It is important to remember that any estimate is of the time when blowfly eggs were laid on the body. Death may have occurred before the insect activity commenced because the body may have initially been inaccessible to the first arriving insects, for instance if the body was wrapped in a blanket or placed in a sealed container such as the boot of a car (Goff, 1993). Clothing, submersion in water or burial can all reduce accessibility of adult insects to a body, and therefore delay or prevent egg-laying. Therefore any post-mortem estimate is of the *minimum* time since death.

6 Case Examples

Case 1

In 2003 in northern England, a man was reported missing, having last been seen on his way home on the evening of 19th November. Despite searching the area, his body was not found until 3rd February 2004, lying at the bottom of a deep ditch, created between a vertical embankment and a single storey building not far from his home. The forensic pathologist estimated that he

had died approximately two to three weeks prior to discovery, i.e. around the middle of January. A forensic entomologist was brought in, and having collected samples of bluebottle larvae and pupae from the body and from the scene, gave an estimated most likely date of insect infestation (minimum time of death) of 27th to 28th November, with a broader range of 19th November to 6th December. So why had the two experts given post mortem intervals differing by seven weeks? The pathologist had based their estimate on standard post mortem changes, bearing in mind the average temperatures at that time of year. However, post mortem changes are highly variable and their use in establishing a time since death has been described as "*an achilles heel in Forensic Medicine"* (Henssge *et al*., 1988), especially more than three days post mortem. The forensic entomologist placed a temperature datalogger at the scene of discovery, i.e. in the ditch, and conducted a regression analysis for the ditch temperatures with those from a nearby meteorological station for the same time period. From this he had been able to estimate the temperatures in the ditch, from the time when the man was last seen alive. It became apparent that the ditch was shielded from direct sunlight and had its own mini-climate, so that the temperatures at which the flies had been developing were overall much lower than would have been expected from the average ambient temperatures. It later transpired that the man had erred from the path on his way home, fallen down the slope and broken his spine, dying instantly – a tragic accident.

Case 2

In July 1999, the badly burnt body of a man was discovered by some children in an old ammunition bunker in Essex. Because of the extensive damage incurred by the body from the fire, which had apparently taken place in the room, the forensic pathologist had great difficulty in estimating a time of death in this case, which was eventually shown to be a suicide. There were large numbers of fly eggs and larvae on the body, so a forensic entomologist was called to the scene. He found no evidence of burnt insects on or around the body, so concluded that adult flies had laid eggs on the

body after the burning had occurred. The temperature in the underground bunker was buffered from ambient fluctuations to a great extent and was relatively constant at about 14°C. The larvae were measured and several distinct size cohorts were found, representing larvae that had hatched from eggs laid on several consecutive days. The largest and, therefore, oldest larvae were estimated to have been laid as eggs six days before the body was found, establishing a minimum time of death. That information enabled the police to focus their investigation to a particular time frame and subsequent witness interviews recorded that a fire had been seen in the bunker on the night before the flies found the body.

Case 3

In April 2003, a body was found burning in an area of open woodland in Southern England. It turned out to be the body of a young woman who had disappeared more than a month before. Forensic examination of the body showed that although it was partly decomposed, there was no evidence of insect activity on or around the body. The forensic entomologist who was consulted concluded that the lack of insect evidence indicated that the body had been stored in a location where flies could not gain access to it. Although seemingly obvious, this information was of value because a body exposed at that time of year, either inside or outside, would have become infested by blowflies if it had not been concealed in some way. This information fitted other evidence that the victim's body had been securely wrapped since death and it later transpired that the body had also been stored inside a large cardboard box within a rented secure storage unit.

These three cases illustrate situations where abnormal conditions made it difficult for the forensic pathologist to estimate a post-mortem interval based on normal decomposition rates. In the first two cases, the blowfly larvae feeding on the bodies could be collected as evidence, and having correctly identified them, and calculated the temperatures at which they developed, it was possible to calculate an accurate minimum post mortem interval.

However, in the third case, it was the *lack* of insect evidence which was important, supporting the prosecution case concerning the post mortem storage of the victim's body. Thus forensic entomology played a significant role in the investigation and solving of these three cases.

7 Conclusion

The objective of this article is to raise further awareness of the application of forensic entomology in criminal investigations. The research carried out at the Anthropological Research Facility in Tennessee presents a unique opportunity to witness the process of decomposition of a human cadaver first hand, and this in turn allows us to use this opportunity to study the vital role that insects play in this process. Although used intermittently in the UK for the past 50 years or so, forensic entomology is now gaining much greater acceptance in this country, and should be viewed as a standard tool to aid criminal investigations where necessary. In particular, the integrity of the science must be maintained by rigorous scientific research, the results of which can be used to carry out accurate and constructive casework, making forensic entomology a valuable asset to the Criminal Justice System in the UK.

Acknowledgements

The authors wish to thank the Winston Churchill Memorial Trust for providing funding for Andrew Hart, the Forensic Science Service® and the Natural History Museum for their support, the staff at the Forensic Anthropology Centre (Dr William Bass, Dr Murray Marks, Dr Richard Jantz, Dr Lee Meadows Jantz) and Dr Ian Dadour (University of Western Australia). We are grateful to the West Yorkshire, Essex and Sussex Police Forces for permission to refer to cases 1, 2 and 3 respectively.

References

Amendt, J. et al (2007) *Best practice in forensic entomology – standards and guidelines. Int. J. Legal Med.* 121: 90-104.

Anderson, G. (2001) Forensic Entomology in British Colombia: A brief history, *Soc Brit Columbia* 98, pp 127-135.

Byrd, J.H. and Castner, J.L., eds. (2001) *Forensic Entomology. The Utility of Arthopods in Legal Investigations.* Boca Raton, Florida: CRC Press.

Catts N.H. and Haskell, N.H. eds. (1990) *Insects and Death: A Procedural Guide*. Clemson, South Carolina: Joyce's Print Shop Inc.

Dadour, I.R, et al (2001) Forensic entomology: application, education and research in Western Australia, *Forensic Science Int,* 120, pp 48-52.

Donovan, S. et al (2006) Larval growth rates of the blowfly, Calliphora vicina, over a range of temperatures. *Medical Veterinary Entomol*. 20, pp 106-114.

Goff, M.L. (1993) Estimation of postmortem interval using arthropod development and successional patterns. *Forensic Sci. Rev* 5, pp 81-94.

Greenberg, B. (1991) Flies as forensic indicators. *J. Med. Entomol*. 28, pp 565-577.

Henssge, C. Madea, B. and Gallenkemper, E. (1988) Death time estimation in case work. II. Integration of different time methods. *Forensic Science Int 39*, pp 77-87.

Marks, M.K. (1995) William M. Bass and the development of forensic anthropology. *J. Forensic. Science* 40, pp 741-750.

Payne, J.A. (1965) A summer carrion study of the baby pig *Sus scrofa* Linnaeus. *Ecology* 46, pp 592-602.

Rodriguez W.C. and Bass, W.M. (1983) Insect activity and its relationship to decay rates of human cadavers in east Tennessee. *J. Forensic Science* 28, pp 423-432.

Rodriguez, W.C. and Bass, W.M. (1985) Decomposition of buried bodies and methods that may aid in their location. *J. Forensic Sci*ence 30, pp 836-852.

Smith, K.G.V. (1986) *A Manual of Forensic Entomology. London, the Trustees of the British Museum (Natural History).* London and Cornell University Press [Ithaca].

Turner, B. (1987) Forensic entomology: Insects against crime. *Science Prog. Oxford* 71, pp 133-144.

ARTICLE

Figure 1. Blowfly larval mass (A. Hart)

Forensic Entomology

Insect activity and its role in the decomposition of human cadavers

***Andrew J. Hart*[1]**
and
***Amoret P. Whitaker*[2]**
(formerly Brandt)

[1] The Forensic Science Service®, 109 Lambeth Road, London SE1 7LP, UK.

[2] The Natural History Museum, Cromwell Road, London SW7 5BD, UK.

Biosketch

Andrew J. Hart (Pic: M. Bordas) is a forensic scientist working at the Forensic Science Service® (FSS) who specialises in the examination of biological evidence. Prior to this, he undertook a PhD at the University of Birmingham and worked as an applied entomologist for over five years. In 2005 he was awarded a Winston Churchill Fellowship, which enabled him to travel throughout the USA and Canada researching forensic entomology (Table 1), including a three week secondment at the Anthropological Research Facility, University of Tennessee, Knoxville, USA.

Amoret P. Whitaker (Pic: M. Hall) is studying for a PhD in forensic entomology with King's College London, based at the Natural History Museum, and also works as a consultant on forensic cases. She undertook a three week research project at the Anthropological Research Facility, Knoxville in 2004.

Introduction

Forensic entomology is the study of insects and other arthropods in a legal context, most commonly to assist in criminal investigations by the interpretation of insect evidence in cases of untimely death (Anderson, 2001; Byrd and Castner, 2001; Greenberg, 1991; Smith, 1986), but it may also be applied in cases of food contamination, illegal importation of goods, fraud, delusory parasitosis, wildlife poaching, and neglect of the young or the elderly. The key aspect of forensic entomology in murder investigations is the estimation of the minimum time since death or post-mortem interval (PMI) (Anderson, 2001; Dadour *et al* 2001; Goff, 1993), which requires an understanding of the taxonomy, physiology and ecology of the insects involved.

The cadaver forms a rich resource, which attracts a succession of insects and other arthropods in a relatively predictable order. These may be necrophagous, predatory, parasitic, omnivorous or adventitious species, and may include Diptera (flies), Coleoptera (beetles), Lepidoptera (butterflies and moths), Hymenoptera (wasps, bees and ants) and Arachnids (spiders) among other groups (Turner, 1987). The first two orders comprise the most frequent visitors, as both immatures and adults. However, the Calliphoridae or blowflies (bluebottles and greenbottles) are the key group of forensically important insects (Greenberg, 1991) because they are the most commonly found insects on dead bodies and they are usually the first insects to arrive at a cadaver, therefore they are the most useful group of insects for estimating the post mortem interval.

Adult blowflies are attracted to the body by the odours produced during the early stages of decomposition. They feed on the body and the females will oviposit (lay eggs) in moist, dark places, primarily the natural orifices of the body (eyes, ears, nose, mouth and genital region), but eggs may also be found in the hair, between the body and the substrate it is lying on, in the folds of clothes, or in open wounds. In the latter case, the presence of eggs or larvae may indicate the presence of a stab or gunshot wound.

The eggs hatch out into first stage (instar) larvae and moult through two more stages. In the late second and throughout the third stage they form larval masses ('maggot masses') of congregative feeding (Figure 1), which increases the larval mass temperature, sometimes up to 20 degrees centigrade above the ambient temperature. This in effect produces a stable optimal heat level for accelerated larval development (Catts and Haskell, 1990). When the larvae have finished feeding, they disperse away from the body to find a suitable pupation site. Depending on the soil substrate, they may burrow into the ground, or move under stones, logs and vegetation. The outer cuticle of the post-feeding larva constricts, hardens and darkens, and over a period of time the pupa inside metamorphoses into an adult fly, which will emerge and begin the life cycle again.

In an ideal situation, a forensic entomologist is called to attend the crime scene to collect insect evidence, for three reasons. Firstly, they are the most qualified person to recognise insect evidence; eggs and early instar larvae may be small, hidden or inconspicuous and therefore easily overlooked, and the oldest specimens may have already left the body to pupate. Secondly, they can record the context in which the evidence was collected, through comprehensive notes and photographs. Thirdly, they can be sure that the evidence has been collected and preserved in the correct way to enable accurate analysis. Calliphorid eggs and adults can be placed directly into ethanol (alcohol), but larvae should first be placed into freshly boiled water to extend them and prevent bacterial decomposition, before placing them in ethanol. Preserved larvae are taken back to the laboratory where the species, instar and age can be determined and live larvae can be bred through to adults in the laboratory to confirm identification. Pupae can be dissected to determine their stage of development.

Figure 2. Decomposition of a pig with associated larval masses (A. Hart)

The developmental rates of insects are dependent on external temperatures. Insects develop within a narrow range of temperatures, the lower limit being the temperature below which no development occurs (the developmental threshold) and the upper limit being the lethal temperature (Hart *et al.*, 1997). Therefore, it is essential that the temperatures at which the insects have developed are calculated. A forensic entomologist places a datalogger at the crime scene to collect ambient temperatures for seven to ten days. Temperature data for the same period is obtained from the nearest meteorological station to the crime scene, and also for the preceding period during which the body may have been there. A regression analysis is carried out on the two locations for the same time period, and based on this, the temperatures to which the developing insects were exposed before the body was found can be estimated. Once the species of fly has been identified, the age of the collected samples can be determined by measuring the length of the larvae or dissecting the pupae, and comparing their stage of development with published data in the context of the temperatures at which they developed. It is important to remember that estimation of the PMI is from when oviposition first occurred; death may have occurred before the insect activity commenced because the insects may have been prevented access to the body until later, for instance if the body was in a sealed container such as the boot of a car or wrapped in a blanket (Goff, 1993). Burial, submersion and clothing can all reduce accessibility of the adult insects to the body, and therefore delay or prevent oviposition. In cases of neglect, death could have occurred later if the infestation was initially a case of myiasis (the larval infestation of live bodies) (Hall and Wall, 1995).

Insect activity and decomposition

Insect activity has a major role to play in the decomposition of a cadaver. Payne (1965) found that carcasses of piglets exposed to insect activity lost 90 per cent of their body weight in six days, whereas carcasses which were free of insects maintained 20 per cent of their original weight after 100 days. Although decomposition is a continuous process, attempts have been made to divide it into a number of stages. Mégnin (1894) identified eight waves of faunal succession in the decomposition of a human cadaver. Goff (1993) recognised five stages of decomposition, each of which attracts different groups of insects. During the *fresh stage* blowflies are attracted to the body to feed and lay eggs. The *bloat stage* occurs because of the release of gases from the rapidly reproducing anaerobic bacteria in the body. The metabolic activity of feeding larvae causes an increase in the temperature of the body, and the leakage of fluids and ammonia makes the local soil alkaline and repels the usual soil-living invertebrates. The frenzied feeding activity of the larval masses allows the gases to escape, resulting in deflation of the body during the *decay stage*. By this stage adult and

Figure 4.

Figure 3.

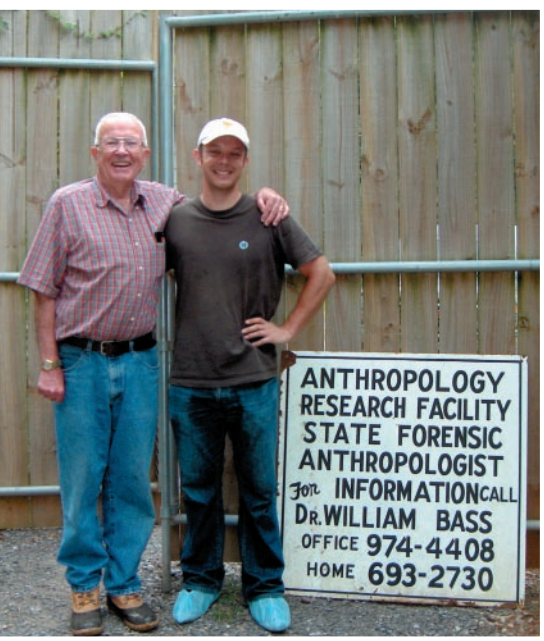


Figure 5.

immature beetles are also present, feeding on the dried tissues of the body and predating upon the fly larvae, which have started leaving the body to pupate. Most of the flesh of the body has been removed before entering the *post decay* stage, leaving skin and cartilage on which beetles are feeding, and bones. Finally, in the *skeletal stage*, all that remains of the body are the bones and hair, with few or no carrion insects present except a recolonisation of soil invertebrates.

The majority of forensic entomology research has used pigs as models for humans, for ethical, economical and physiological reasons: a) they are already bred for the food industry; b) they are therefore cheap and easy to acquire; c) stillborn piglets may be used; and d) they have a fairly hairless body and similar internal organs (Figure 2). However, there is one location in the world where it is possible to study the decomposition process in human cadavers, namely the Anthropological Research Facility, University of Tennessee, Knoxville, USA.

The Anthropological Research Facility, University of Tennessee, Knoxville, USA

The Anthropological Research Facility was founded in 1981 by Professor William 'Bill' Bass (Figure 3), a pioneer of forensic anthropology, in order to further the understanding of physical anthropology. Colloquially called 'The Body Farm', a name coined by the crime author Patricia Cornwell as the title of one of her novels, the Facility is a 1.3 acre site situated on a wooded hillside close to a hospital just outside of Knoxville (Figure 4). The Facility serves two main purposes: firstly to enable the study of the decomposition process of human bodies, and secondly, to accession the remains into the department's William M. Bass Donated Skeletal Collection. Approximately 80 to 100 bodies are donated each year, about 75 per cent by the families, the rest being from medical schools, unclaimed bodies from the police, or self-donations arranged prior to death. Once transported to the Facility, the bodies are laid out on the ground, sometimes in their body bags and sometimes behind low wire fences to prevent scavengers such as raccoons from scattering the remains. During this time, students of the department and invited scientists can study the decomposition process and carry out scientific studies. For this reason, some bodies may be placed in various 'crime scene' scenarios, for example in a car boot, placed indoors, partially submerged, buried in the ground or under concrete. Decomposition to the skeletal stage may take six months to a year, after which the remains are cleaned, sorted and added to the department's skeletal collection for future research, teaching and casework. The value of this unique collection is enhanced by the documentation relating to each donor body, which can include details of their gender, race, age, stature, life style and medical history. This information can be referred to in human identification studies, and cranio-facial reconstructions can be accurately assessed as a photograph of the donor, taken when they were alive, is also kept on file. In addition to the significant role which the Facility plays in scientific research, it

is also a unique resource which is used for body recovery training courses by the FBI and international disaster recovery teams.

Entomology studies at the Anthropological Research Facility

Few entomologists have had the opportunity to visit and work at the Facility, and unsurprisingly there is a sparsity of research in the area of insect activity and human decomposition (Rodriguez and Bass, 1983; 1985). Collaborative research has been carried out by scientists from the Forensic Science Service® and the Natural History Museum, which involved the study of insect activity in relation to the decomposition of four human cadavers. Among the factors investigated were insect attraction, selection of oviposition sites, larval development, formation of larval masses, post-feeding larval dispersal, selection of pupation sites, and the effect of reduced accessibility on insects and the resulting affect on decomposition.

Twice a day over a three week period, insect samples were collected including eggs, larvae, pupae and adults that were present on and around the bodies. These samples were retained for analysis and to create a voucher collection, and a proportion of the immature specimens collected were reared through to adulthood in order to assist in the identification of the insects. Internal body temperatures were monitored using either Tinytalk® or Tinytag® datalogger probes inserted into the mouth, rectum and torso of the cadavers, and the ambient temperatures were also recorded. In addition, the temperatures of the larval masses that formed were measured using a non-invasive infra-red thermometer. At the maximum feeding stage, these showed an increase of 20 degrees centigrade above ambient, with temperatures reaching over 45 degrees centigrade. Photographs were taken twice a day for three weeks, recording the decomposition of the cadavers. A written record of the insect activity and state of decomposition was kept, including details of the post-feeding dispersal of larvae away from the bodies being studied plus three additional bodies. Large quantities of larvae were observed dispersing away from each donor, sometimes following narrow trails, sometimes in large 'carpets' (Figure 5), before they buried into the soil to pupate. Despite various commonly held assumptions, the larvae did not always move downhill, nor did they simply radiate in all directions. In one case significant larval dispersal was recorded on seven consecutive days.

Decomposition itself consists of a number of processes including the enzymatic liquefaction of cells, bacterial decomposition of tissue, the drying of the skin and remaining soft tissue, and finally skeletonisation. The rate at which this occurs depends on environmental conditions, particularly temperature and humidity, as well as decomposer activity. In the summer months at the Facility, it was possible to witness almost the whole process of decay from fresh through to skeletal within three weeks, as the temperature and humidity during July and August in Tennessee provided optimum conditions for bacterial and insect activity. For instance, a female donor body rapidly progressed from fresh to almost skeletal in only nineteen days in August, 2005. This was almost entirely due to the large amount of insect activity, with tens of thousands of larvae forming substantial larval masses on the body, predominantly of the Calliphoridae species *Cochliomyia macellaria* F. (secondary screwworm fly) and *Phormia regina* (Meigen) (black blowfly).

Itinerary

- Florida – Attendance at the North American Forensic Entomology Association (NAFEA) conference (20th – 22nd July)
- Texas – Study of the decomposition of a dead goat with Dr Jeff Tomberlin and Dr Jim Olson, Texas A&M University (25th – 29th July)
- West Virginia – Identification of necrophagous insects using DNA techniques with Dr Jeff Wells, West Virginia University (1st – 5th August)
- Tennessee – Three-week research study on insect succession on two human donor bodies at the Anthropological Research Facility, Knoxville (8th – 26th August)
- Indiana – Case work and pig decomposition with Dr Neal Haskell, St Joseph's College (29th August – 2nd September)
- Vancouver – Calculation of PMI and underwater pig decomposition with Dr Gail Anderson, Simon Fraser University (5th – 9th September)
- Hawaii – Application of Forensic Entomology in Hawaii with Dr M. Lee Goff, Chaminade University (12th – 16th September)

Table 1. Full itinerary of a Fellowship to study forensic entomology in the USA and Canada. Dr Andrew Hart was awarded a Winston Churchill Memorial Trust Travel Fellowship to spend eight weeks during the summer of 2005 meeting and working with many of the top practising forensic entomologists in North America.

Because the Anthropological Research Facility in Tennessee is the only place in the world where the process of decomposition of human cadavers can be studied, and because there is no guarantee that donor bodies will be available during any planned research period, future visits are planned in order to gather enough data to ensure sufficient replication.

Conclusion

Forensic entomology is becoming more commonly used in the investigation of serious crimes in the UK, and the increasing amount of research being undertaken in this field reflects this, especially in the USA and Canada. The research carried out at the Facility provided a unique opportunity to study the processes involved in the decomposition of human cadavers and the vital role that insects have in this process. The data that has been collected will go on to form part of a larger study of insects and decomposition, with additional replicates to increase the robustness of the results so far. Ideally, additional research centres like the Anthropological Research Facility need to be established worldwide in order to assess differences in the relationships of insects and decomposition in different geographic and climatic locations. There is a real need for fundamental research such as this in the field of forensic entomology. Although a fascinating subject in itself, ultimately it is a valuable tool which can be used to aid the police in criminal investigations.

Acknowledgements

Andrew Hart wishes to thank the Winston Churchill Memorial Trust for providing funding, the Forensic Science Service® for their support, and his hosts in the USA and Canada who included Dr Jeff Tomberlin, Dr Jim Olson, Dr Mariana Griggs, Detective Bill Pepsis, Dr Jeff Wells, Christine Picard, Dr Richard Jantz, Dr Lee Meadows Jantz, Dr Murray Marks, Dr Bill Bass, Dr Steven Symes, Dr Anne Kroman, Dr Neal Haskell, Joel Haskell, Dr Gail Anderson, and Dr M. Lee Goff. Amoret Whitaker thanks the staff at ARF (Dr Bill Bass, Dr Murray Marks, Dr Richard Jantz, Dr Lee Meadows Jantz) and Dr Ian Dadour (University of Western Australia). Both authors thank Dr Martin Hall from the Natural History Museum for his help and advice.

References

Anderson, G. (2001). Forensic Entomology in British Colombia: A brief history. *J. Entomol. Soc. Brit. Columbia* **98**: 127-135.

Byrd J.H. & Castner, J.L., eds. (2001). *Forensic Entomology. The Utility of Arthopods in Legal Investigations.* Boca Raton, Florida: CRC Press.

Catts N.H. & Haskell, N.H. eds. (1990). *Insects and Death: A Procedural Guide.* Clemson, South Carolina: Joyce's Print Shop Inc.

Dadour, I.R, Cook, D.F., Fissiloi, J.N. and Bailey, W.J. (2001). Forensic entomology: application, education and research in Western Australia. *Forensic Sci. Int.* **120**: 48-52.

Goff, M.L. (1993). Estimation of postmortem interval using arthropod development and successional patterns. *Forensic Sci. Rev* **5**: 81-94.

Greenberg, B. (1991). Flies as forensic indicators. *J. Med. Entomol.* **28**: 565-577.

Hall, M.J.R. and Wall, R. (1995). Myiasis of humans and domestic animals. *Adv. Parasitol.* **35**: 257-334.

Hart, A.J., Bale, J.S, and Fenlon, J.S. (1997) Developmental threshold, day-degree requirements and voltinism of the aphid predator *Episyrphus balteatus* (Diptera: Syrphidae). *Ann. Appl. Biol.* **130**: 427-437.

Mégnin, J.P. (1894) *La faune des cadavers: application de l'entomologie à la medicine légale.* Encyclopédie scientifique des Aide-mémoires, Masson et Gauthier-Villars, Paris.

Payne, J.A. (1965). A summer carrion study of the baby pig *Sus scrofa* Linnaeus. *Ecology* **46**: 592-602.

Rodriguez W.C. and Bass, W.M. (1983). Insect activity and its relationship to decay rates of human cadavers in east Tennessee. *J. Forensic Sci.* **28**: 423-432.

Rodriguez, W.C. and Bass, W.M. (1985). Decomposition of buried bodies and methods that may aid in their location. *J. Forensic Sci.* **30**: 836-852

Smith, K.G.V. (1986). *A Manual of Forensic Entomology.* London, the Trustees of the British Museum (Natural History). London and Cornell University Press [Ithaca].

Turner, B. (1987). Forensic entomology: insects against crime. *Sci. Prog. Oxf.* **71**: 133-144